

TISSUE CULTURE LAB MANUAL (BIOT 77 IVC)



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This text was compiled on 10/11/2026

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Licensing

A detailed breakdown of this resource's licensing can be found in **Back Matter/Detailed Licensing**.

1: Standard Operating Procedures (SOP) and Documentation

Learning Objectives

- Identify an SOP's purpose, scope, responsibilities, and required controls.
- Distinguish a current approved procedure from an obsolete revision.
- Apply ALCOA+ principles to entries, corrections, and deviations.
- Evaluate whether a completed record allows another person to reconstruct the work.

BACKGROUND

A **standard operating procedure (SOP)** (an approved written set of instructions for a recurring task) helps trained personnel perform work consistently. In a **current Good Manufacturing Practice (cGMP)** environment (a manufacturing system governed by requirements for product quality and control), procedures and records support reliable production and investigation of problems. In this exercise, you will practice those habits by reviewing an SOP, recording a simple task, and checking the resulting record.

An SOP's **purpose** (the reason the procedure exists) explains the task's intended outcome, while its **scope** (the activities and personnel covered by the procedure) sets its boundaries. A cabinet-cleaning SOP, for example, identifies which cabinets and activities it covers. It should describe cleaning and disinfection accurately rather than promise a sterile workspace. Responsibilities identify who performs the work, who reviews the record, and who may approve changes. **Quality assurance (QA)** (oversight that helps ensure processes meet defined requirements) may review procedures and records within the organization's quality system.

Instructions should identify required materials, equipment, safety precautions, sequence, and criteria for acceptable completion. **Version control** (identification and management of the current and earlier document revisions) helps prevent use of obsolete instructions. **Change control** (formal assessment and approval of proposed changes) determines when revised instructions may take effect. A **deviation** (a departure from an approved instruction or expected condition) must be recorded and handled through the defined process. A missing reagent does not authorize a technician to choose a substitute independently; stop the affected work and obtain the required direction.

Documentation Integrity

Data integrity (the completeness, consistency, and accuracy of data throughout its life cycle) allows another person to reconstruct what happened. The **ALCOA+ principles** (attributes used to assess whether records are trustworthy) apply to paper and electronic records. They guide how observations, calculations, corrections, and unexpected findings should be captured.

Attributable (linked to the person who performed the action): identify the operator and use an individual login for electronic entries.

Legible (readable and understandable): record clear text, units, and identifiers.

Contemporaneous (recorded when the activity occurs): enter the actual observation at the time of work. Identify a late entry honestly rather than backdating it.

Original (the first record or a verified true copy that preserves its meaning): retain the source data and relevant context. Recopying selected values into a clean sheet does not replace the source record.

Accurate (a truthful representation of the activity or measurement): report the observed value and follow the approved precision and rounding rules.

Complete (containing all required data): retain unexpected results, errors, and repeat measurements with explanations.

Consistent (following a logical sequence and established conventions): use the required units, date format, and time format.

Enduring (preserved in a durable record): use approved paper or electronic systems and retention practices.

Available (retrievable for authorized review when required): store records where responsible personnel can access them.

For a paper correction, keep the original entry readable, add the correct information, and identify and date the correction according to the documentation SOP. Add a reason when required or when it is not evident. Do not erase, cover, or discard the original record. Electronic changes should preserve the appropriate **audit trail** (a record showing who changed data, when, and what changed).

MATERIALS

- Current approved SOP for an instructor-assigned recording task
- Current and obsolete SOP revisions supplied for comparison
- Documentation SOP, laboratory record sheet, and deviation form
- Permanent-ink pen and timer or clock
- Instructor-provided data or materials for the assigned task

METHODS

1. Select the correct SOP. Compare the versions provided by the instructor and identify the current approved version that is in effect. Record its document number, revision number, and effective date. Ask the instructor to clarify any missing information before proceeding.

2. Review the procedure. Read the SOP from beginning to end. Identify its purpose, the tasks it covers, your responsibilities, the required materials, and the criteria for completing the work. Resolve any unclear or conflicting instructions with the instructor.

3. Prepare your record. Enter your name or assigned operator ID, the date, and the task on the record sheet. Add the material and equipment identifiers required by the SOP. Use the approved writing and date formats.

4. Perform and document the task. Follow the SOP in order. Record each observation or measurement when it occurs, including units and times when required. Show calculations and retain the original data. Record unexpected results as well as expected results.

5. Correct entries when needed. Follow the documentation SOP. For a paper entry, draw a single line through the error so it remains readable, enter the correct information, and add your initials and the date. Include a reason when required or when the change is not self-explanatory. If an entry was missed, identify it as a late entry and record the actual entry date; do not backdate it.

6. Document a deviation. Review the scenario supplied by the instructor. On the deviation form, describe what occurred, when it occurred, and which materials, steps, or data were affected. Record who must be notified and the next action authorized by the instructor or SOP. If a deviation occurs during your task, stop the affected work and obtain direction before continuing.

7. Review a partner's record. Exchange records and check them against the ALCOA+ principles in the Background section. Confirm that entries identify the operator, are readable, include required data, and show the sequence of work. Note missing or unclear information on the review sheet; do not change your partner's entries.

8. Address review findings and submit. Return the record to its author to address questions according to the documentation SOP. Preserve the original entries and identify any corrections or late additions. Submit the completed record, original data, calculations, review sheet, and deviation form to the instructor using the assigned submission or storage location.

EXERCISE Standard Operating Procedures (SPO) and Documentation NAME_____

EXPECTATIONS

1. Predict which details a reviewer will be unable to confirm if observations are entered from memory at the end of the task.

2. Predict how using an obsolete SOP could affect the recorded procedure or outcome.

RESULTS

Submit the identified SOP revision, completed task record, calculations, any correction or deviation, and the partner review. Identify specific entries supporting each ALCOA+ attribute and record any unresolved question.

Student: _____ Partner: _____ Date: _____

Record units with measurements. Use N/A for items not measured. Attach raw data as needed.

Document and task record

SOP ID / revision	Task / record ID	Date / time	Operator / reviewer

Task measurements

Step / item	Expected value / units	Actual value / units	Time / initials

ALCOA plus review

Attribute	Specific entry / evidence	Issue / action
Attributable		
Legible		
Contemporaneous		
Original		
Accurate		
Complete		
Consistent		

Enduring		
Available		

Corrections and deviations

Record / entry	Issue / correction	Reason / action	Initials / date

Calculations

Observations deviations and corrective actions

Data images graphs or record attachment IDs: _____

CONCLUSIONS

1. Which evidence shows that the correct SOP revision was used? Explain any uncertainty.
2. How well does the record satisfy ALCOA+? Use two entries to support your assessment.
3. Were corrections and deviations handled so that the original information and sequence remain clear? Explain.

4. What information would a new operator or reviewer still need, and how would you improve the procedure or record?

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2: Aseptic Technique and Biosafety Cabinets

Learning Objectives

- Explain how Class II BSC airflow supports aseptic handling.
- Prepare and organize a cabinet according to its operating instructions.
- Perform a liquid transfer while protecting sterile surfaces.
- Recognize a technique error and document the response.

BACKGROUND

Aseptic technique (practices that prevent contamination of materials during handling) relies on intact sterile supplies, careful movement, and appropriate workspace preparation. A **Class II biosafety cabinet (BSC)** (a ventilated enclosure designed to provide personnel, product, and environmental protection during appropriate biological work) uses controlled airflow and **HEPA filtration** (high-efficiency filtration that removes airborne particles). Blocking a grille or making rapid movements can disrupt protection. A clean bench and a BSC provide different kinds of protection and must not be assumed interchangeable.

Contamination can enter through a touched pipette tip, exposed bottle opening, dirty item exterior, or interrupted airflow. Once introduced, microorganisms can multiply in nutrient-rich medium and alter cell growth or experimental results. A vessel may look clear immediately after a handling error, so appearance at the end of a transfer cannot establish freedom from contamination.

Disinfection (reduction or inactivation of microorganisms by an appropriate treatment) requires an agent suitable for the target organisms and surface. **Contact time** (the period a disinfectant must remain in contact with a surface to act as specified) matters as much as the choice of agent. **Sterilization** (a validated process that eliminates viable microorganisms, including resistant forms) is different. Surface wiping and glove alcohol treatment reduce contamination risk but do not establish sterility.

Verify the cabinet's operating status and use the sash height, startup time, and work position specified for that model. Organize supplies from clean to used, keep airflow grilles clear, and avoid reaching over open vessels. Choose a left-to-right or right-to-left arrangement that fits the task; the direction itself is not a universal rule. Disinfect surfaces and compatible item exteriors using the laboratory-approved agent and its required contact time. Alcohol is not appropriate for every organism or spill. Ultraviolet (UV) lamps do not replace cleaning and chemical disinfection and should not be used as a routine sterilization step. Follow the institution's cabinet policy and keep any UV source off during work.

Set up the cabinet so that clean supplies, the working area, and waste have clear positions. Open each vessel only when needed, keep pipette tips and vessel openings away from unapproved contact, and avoid moving used items back through the clean area. A **deviation** (a departure from an approved instruction or expected condition) such as touching a protected surface should trigger the response specified by the laboratory procedure. Record the event and response so its possible effect can be assessed.

MATERIALS

- Current approved SOPs for documentation, PPE, BSC operation, cleaning, and waste handling
- Certified Class II BSC with model-specific operating instructions
- PPE required for the assigned task and handwashing facilities
- Laboratory-approved disinfectant, compatible wipes, and contact-time instructions
- Sterile pipettes or tips, closed sterile vessels, and approved medium or practice liquid
- Labeled waste container and the approved aspiration setup, if used
- Laboratory record sheet, permanent-ink pen, and deviation form
- Timer and cabinet or equipment identification information

METHODS

1. Review the assigned procedures. Read the current approved SOPs for cabinet operation, cleaning, waste handling, and the liquid transfer. Record their document numbers and revisions. Identify the required PPE, disinfectant, contact time, cabinet settings, and transfer volume. Ask the instructor to clarify any missing or conflicting instruction.

2. Prepare your supplies and record. Gather only the materials needed for the transfer. Label the receiving vessels as directed. Enter your name, the date, the task, and the cabinet ID on the record sheet. Record observations and required times as the work proceeds.

3. Don the required PPE. Perform hand hygiene and put on PPE in the approved sequence. Check garment fit and glove integrity. Have a partner or instructor verify the required coverage and record any correction before beginning.

4. Check and start the cabinet. Verify the certification status and set the sash at the marked operating height specified for that model. Start the blower and work light as directed, allow the required startup interval, and check airflow indicators and alarms. Keep any UV lamp off during work. Resolve an alarm with the instructor before proceeding.

5. Clean and disinfect the work area. Follow the cleaning SOP for accessible work surfaces. Apply the approved disinfectant using the specified method and keep surfaces wet for the required contact time. Keep airflow grilles clear and avoid flooding them. Record the disinfectant and completion time.

6. Bring in and arrange materials. Disinfect compatible item exteriors before placing them inside the cabinet. Arrange clean supplies, the transfer area, and waste in a consistent flow from clean to used. Keep front and rear grilles unobstructed and avoid unnecessary supplies.

7. Prepare for aseptic handling. Disinfect compatible gloves as required by the SOP and allow them to dry. Position the work at the prescribed distance inside the cabinet. Move deliberately, open vessels only when needed, and keep hands and sleeves from passing over exposed sterile openings.

8. Perform the liquid transfer. Use the sterile pipette or tip specified for the assigned volume. Keep tips and vessel openings from touching unapproved surfaces, and close vessels promptly after the transfer. Record the material identity, volume, and any observed contact or handling error.

9. Respond to a handling error. If a glove, tip, or protected surface contacts a potentially contaminated item, stop the affected operation. Follow the SOP to replace or disinfect affected items and obtain direction from the instructor as needed. Record the event, affected materials, and corrective action. Use the approved spill procedure if a spill occurs.

10. Clean and finish cabinet work. Close all vessels and remove materials through the approved workflow. Handle waste as directed, then clean and disinfect the work surfaces using the required contact time. Follow the cabinet-specific purge and shutdown instructions. Record completion and any unresolved issue.

11. Remove PPE and complete the record. Doff PPE in the approved sequence, avoiding contact with exposed outer surfaces. Place disposable and reusable items in their designated locations and perform hand hygiene. Review the record for cabinet checks, transfer details, and corrective actions, then submit it to the instructor.

EXERCISE LAB TITLE NAME_____

EXPECTATIONS

1. Predict how blocking a cabinet grille or reaching over an open vessel could affect contamination risk during the transfer.
2. Predict how touching a sterile pipette tip to an unapproved surface would affect your confidence in the transferred material.

RESULTS

Submit the cabinet checks, disinfectant and contact time, workspace arrangement, transfer identities and volumes, handling observations, and any deviations or corrective actions. Record actual observations separately from explanations. If no microbiological test was performed, do not assign a sterility result.

Cabinet preparation record

Student: _____ Partner: _____ Date: _____

Task: _____ Cabinet ID and model: _____

SOP numbers and revisions: _____

Certification status/date: _____ Sash height: _____

Startup interval: _____ Blower start time: _____

Airflow indicators/alarms: _____ PPE check and corrections: _____

Record units and actual observations. Use N/A for measurements not performed.

Cleaning and disinfection log

Stage or surface	Disinfectant / lot	Required contact time	Actual start / end times

Liquid transfer record

Source ID / material	Receiving vessel ID	Planned / actual volume and units	Time / operator

Handling observations

Student: _____ Partner: _____ Date: _____

Workspace arrangement: sketch or label clean supplies, work area, waste, and grilles below.

Record units and actual observations. Use N/A for measurements not performed.

Technique and response record

Time / step	Observed action or error	Affected material / vessel	Action taken / reviewer

Completion record

Check	Result or details	Time / initials
Vessels closed and removed		

Waste handled		
Final cleaning completed		
Purge / shutdown completed		
PPE removed and hand hygiene completed		

Workspace sketch

Calculations and observations

Record transfer-volume calculations or checks, if needed.

Observations deviations and corrective actions

CONCLUSIONS

1. Which observed actions protected sterile surfaces and cabinet airflow? Use examples from your transfer.
2. Which event created the greatest contamination risk, and was its response appropriate? Explain.
3. How did the model-specific instructions affect cabinet preparation, work position, and shutdown?

4. What can the observations establish about your technique, and what additional evidence would assess actual contamination?

3: Donning and Doffing Personal Protective Equipment (PPE)

Learning Objectives

- Explain how PPE protects personnel and helps control contamination.
- Select the PPE specified for the assigned task.
- Demonstrate the approved donning and doffing sequences.
- Identify and correct contact errors, damaged gloves, and poor garment fit.

BACKGROUND

Personal protective equipment (PPE) (clothing or devices used to reduce exposure to hazards) also helps limit the material shed from people into the work area. **Donning** (putting on PPE) and **doffing** (removing PPE) require care because contaminated outer surfaces can transfer material to hands or clothing. Classroom laboratory PPE and cleanroom gowning are different: the required items and order depend on the hazards, room, and approved procedure. A lab coat, gloves, and suitable eye protection may be required; masks, hair or beard covers, shoe covers, and sterile coveralls are used when specified. A face shield protects against splashes and does not automatically replace required eye protection or a mask.

People shed skin cells, hair, and droplets during movement. Protective garments limit their transfer, while gloves and eye protection reduce exposure. PPE must match the task; loose cuffs or damaged gloves can leave a contact route.

Gowning (putting on the garments required for a controlled work area) should occur in the designated area and follow the posted sequence. Inspect each item before use and avoid touching protected surfaces with bare hands. A partner can observe places that are difficult to see, such as uncovered hair or gaps at the cuff. Correct the problem before starting the task.

Follow the posted gowning sequence rather than treating one sequence as universal. Perform hand hygiene before donning and after doffing. Keep protected garment surfaces from contacting benches or the floor, inspect gloves, and replace damaged or contaminated items. **Disinfection** (reduction or inactivation of microorganisms using an appropriate treatment) differs from **sterilization** (a validated process that eliminates viable microorganisms, including resistant forms). Applying 70% isopropyl alcohol to compatible gloves, when required by the SOP, disinfects them; it does not make them sterile. Gloves must still be changed when contaminated and at the transitions specified by the laboratory.

During doffing, assume that exposed outer surfaces may be contaminated. Remove items using the approved method, keep those surfaces away from skin and clean clothing, and separate disposable items from items intended for approved cleaning and reuse. **Hand hygiene** (cleaning the hands by the method appropriate to the task) is required before donning and after doffing because gloves can have defects and hands can become contaminated during removal.

MATERIALS

- Approved PPE and gowning SOP for the assigned task
- Required lab coat or coverall, gloves, and eye protection
- Mask, hair or beard cover, shoe covers, or restricted-use footwear when specified
- Handwashing facilities and approved hand-hygiene supplies
- Designated donning and doffing areas and waste containers
- Compatible glove disinfectants when required by the SOP
- Partner observation sheet and permanent-ink pen

METHODS

1. Review the PPE procedure. Read the current approved SOP for the assigned task. Record its document number and revision, the required PPE, the donning and doffing sequences, and the disposal or reuse instructions. Ask the instructor to clarify any missing or unclear direction.

2. Prepare your supplies and record. Enter your name, the date, and the task on the observation sheet. Inspect each PPE item for damage, cleanliness, and correct size. Identify the designated preparation area and containers for disposable and reusable

items.

3. Don the required PPE. Perform hand hygiene, then put on each item in the order specified by the SOP. Keep protected garment surfaces away from the floor and unapproved surfaces. Have your partner record the actual sequence as you proceed.

4. Check coverage and fit. Ask your partner to inspect garment coverage, glove integrity, and cuff overlap as required by the SOP. Adjust or replace items as needed before beginning the task. Record any issue and the action taken.

5. Follow the glove requirements. If the SOP requires glove disinfection, use the specified agent and method on compatible gloves and allow them to dry. Replace damaged or contaminated gloves and perform hand hygiene as directed. Record any glove change or handling error.

6. Complete the assigned handling task. Perform the movement or clean handling task assigned by the instructor. Have your partner observe contact with PPE and surrounding surfaces. If an error occurs, pause, follow the SOP's corrective instructions, and record the event and response.

7. Doff the PPE. Remove each item in the approved sequence, keeping exposed outer surfaces away from bare skin and clean clothing. Place disposable and reusable items in their designated containers. Have your partner record the removal sequence and any contact error.

8. Complete hand hygiene and review the record. Perform hand hygiene after removing PPE. Review the observation sheet with your partner for the actual sequences, fit checks, errors, and corrective actions. Address missing information according to the documentation SOP and submit the completed record to the instructor.

EXERCISE Donning and Doffing Personal Protective Equipment (PPE) NAME_____

EXPECTATIONS

1. Predict how a torn glove or poorly fitting cuff could affect personnel protection or contamination risk.

2. Predict which removal actions are most likely to transfer material from PPE to bare hands or clothing.

RESULTS

Record the required and actual donning and doffing sequences, item condition and fit, partner observations, glove changes, and corrective actions. Report observed technique; do not claim that PPE was sterile solely because alcohol was applied.

Student: _____ Partner: _____ Date: _____

Record units with measurements. Use N/A for items not measured. Attach raw data as needed.

PPE condition and fit

Item	Required?	Condition / fit	Correction / partner check

Donning sequence

Step	Required action	Actual action / time	Partner observation

Doffing sequence

Step	Required action	Actual action / time	Partner observation

Glove changes and corrective actions

Time / step	Reason / observed issue	Action taken	Observer / initials

CONCLUSIONS

1. Did your actual sequences follow the assigned SOP? Explain any departure.
2. Which observed contact or fit issue created the greatest risk, and why?
3. How did your corrective actions improve protection or contamination control?
4. How would the PPE requirements change for a different task or controlled environment? Identify the instructions you would consult.

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4: Batch Records Documentation and Inventory

Learning Objectives

- Distinguish the approved master batch record from the issued record completed for a particular batch.
- Record material, equipment, processing, label, and inventory data as work is performed.
- Calculate material use, label reconciliation, and final yield; document and report discrepancies.
- Evaluate whether the batch record is complete and ready for independent review.

BACKGROUND

A **batch** (a defined quantity of material prepared under one set of instructions) receives a unique **batch ID** (an identifier that distinguishes it from every other batch). In this exercise, you will prepare four tubes of Medium A and document what happened during preparation. A record connects each finished tube to the source material, equipment, person, measurements, and date. This connection is **traceability** (the ability to follow a product and its history through recorded information). If a problem appears later, a reviewer can use the batch ID to identify the affected tubes and investigate their history.

The **master batch record (MBR)** (the approved instructions and required checks for making a batch) describes what should happen. MBR-TC-001 Rev 02 calls for four capped 15 mL tubes of Medium A, each with a **nominal volume** (the intended volume) of 5.0 mL. A **controlled copy** (an identified copy issued for a particular use) is assigned to one batch. When you enter the actual materials, equipment, times, measurements, and observations, it becomes an **executed batch production record (BPR)** (the completed record of what happened for that batch). Comparing the BPR with the MBR helps a reviewer check whether the approved instructions were followed.

Good documentation practices (rules for making records clear, timely, and trustworthy) require you to identify who made an entry, write legibly, record information when the work occurs, and preserve the original entry. Enter what you actually observe, even when it differs from the target. To correct a paper entry, draw one line through the mistake so it remains readable, enter the correction, and add your initials and date according to your instructor's procedure. Do not erase, obscure, or backdate an entry. Mark a field **N/A** (not applicable) only when that field does not apply, and give the reason when the form requires it. Sign only for work you performed; another person must complete the independent review.

Before you dispense, compare each material's name, **lot number** (the identifier for a particular manufactured quantity), expiration date or approved status, and available quantity with the container label and inventory record. Confirm that the measuring device's check is current. These checks help prevent an incorrect material or unsuitable device from being used. Record the identity of the device so every tube can be linked to the measurement equipment. A finished tube meets the **acceptance criteria** (the requirements used to decide if a unit is acceptable) when its measured volume is 4.8–5.2 mL and it is capped, intact, and correctly labeled.

Reconciliation (comparing recorded quantities with the items or material actually present) helps reveal omissions and losses. For labels, labels issued should equal labels applied + labels spoiled or destroyed + labels returned. For Medium A, total use includes material dispensed into accepted and rejected tubes, replacement draws, and separately recorded loss; do not count one draw twice. Expected closing balance = verified opening balance – total documented use. **Variance** (the difference between two quantities being compared) = observed closing balance – expected closing balance. **Yield** (the fraction of planned acceptable units actually obtained) = acceptable tubes ÷ four planned tubes × 100%. Record the numbers and calculations so another person can check them.

A discrepancy is an observation that does not match a requirement or a recorded quantity. A missing label, a tube outside the volume range, or unexplained inventory variance must be documented and reported to the instructor. Do not adjust a number to make the records agree. The instructor may direct you to place affected material **on hold** (set aside so it cannot be used while an issue is assessed). An independent reviewer checks the BPR, calculations, supporting records, and unresolved discrepancies before recording the **batch disposition** (the documented decision about whether the batch may be accepted or must remain on hold or be rejected).

MATERIALS

- MBR-TC-001 Rev 02 and one issued batch production record
- Medium A source container with label and inventory ledger
- Four 15 mL tubes with caps and approved spare tubes, if supplied
- Unit labels or marker and label issue record
- Verified measuring device and its check log
- Clean tube rack
- Discrepancy form and pen with permanent ink
- Appropriate laboratory personal protective equipment

METHODS

1. Obtain one controlled copy of MBR-TC-001 Rev 02. Enter the assigned unique batch ID, copy number, operator identity, location, and actual start date and time in the issued batch record.
2. Perform the Section 5.1 line clearance. Confirm that unrelated materials and labels have been removed. Record the result, date, time, and your identity. Stop and notify the instructor if the area does not pass.
3. Follow Section 5.2 to compare the Medium A, tube, and label identities with their physical labels and inventory records. Record the actual lot numbers, expiration dates or status, and available quantities. Do not use an unidentified, expired, or unapproved item.
4. Follow Section 5.3 to record the measuring device and tube rack IDs. Confirm that the measuring device check is current and acceptable, and cite its check record. Stop if the required evidence is missing.
5. Prepare labels for tubes 1–4 according to Section 6.1: batch ID, Medium A, tube number, nominal 5.0 mL volume, preparation date, and operator ID. Record labels issued, applied, spoiled or destroyed, and returned. Confirm that issued equals applied plus spoiled or destroyed plus returned.
6. Complete Section 6.2. If mixing is required by the local instruction, mix the source as directed and record the time. Otherwise record N/A and the reason.
7. Using the verified device, dispense Medium A into four tubes as directed by Section 6.3. Cap and inspect each tube. Record each actual volume, date and time, label and cap check, and operator ID immediately after completing that tube.
8. Apply the Section 4 acceptance criteria. Segregate any tube outside 4.8–5.2 mL or with a faulty cap or label. Record rejected and replacement units, any additional Medium A used, and the associated discrepancy or attachment.
9. Complete Section 7.1 material reconciliation. Sum all accepted, rejected, and replacement draws. Subtract total documented source use from the verified opening balance. Record the observed closing balance and how it was determined. Calculate observed minus expected balance.
10. Update the inventory record with the material lot, batch ID, quantity used, balances, date and time, and operator. If the difference is unexplained, document it on a discrepancy form, place the source material on hold as directed, and notify the instructor. Do not change the original numbers to force agreement.
11. Complete Section 7.3 yield reconciliation: accepted units divided by four planned units, multiplied by 100. Account for all dispensed tubes. Complete the operator review and sign only for your own work.
12. Submit the issued BPR, associated labels or label record, inventory entry, equipment check reference, and any discrepancy form for independent review. The reviewer completes Sections 9 and 10, including the documented batch disposition.

EXERCISE Batch Records Documentation and Inventory NAME_____

EXPECTATIONS

1. Before beginning, which entries would let a reviewer trace a finished tube to the source lot and measuring device? Explain why each entry is needed.

2. If the observed closing balance is lower than the calculated balance, what explanations would you consider, and what evidence would you need before choosing one?

RESULTS

Complete or attach the issued BPR and inventory entry. Include the actual batch ID; material and tube lot numbers; equipment ID and check reference; label counts; the measured volume and acceptance decision for every tube; total Medium A use; expected and observed closing balances and variance; accepted and rejected tube counts; percent yield; and any discrepancy references. Show your calculations. Leave no required field blank: follow the record instructions for fields that do not apply. Record actual measurements rather than copying the target values.

Batch preparation record

Student: _____ Partner: _____ Date: _____

Use these pages to organize exercise data. Complete the issued BPR and required supporting records as directed.

Batch ID: _____ MBR revision: _____ Controlled copy number: _____

Location: _____ Operator ID: _____ Start date/time: _____

Line clearance result/time: _____ Device ID/check reference: _____

Tube rack ID: _____ Mixing time or N/A reason: _____

Record units and actual observations. Use N/A for measurements not performed.

Material verification

Material	Lot / identity	Expiry / approved status	Verified available quantity and units
Medium A			
Tubes			
Labels			

Tube filling and acceptance

Tube ID / original or replacement	Actual volume (mL)	Cap / integrity / label checks	Accept or reject / reason	Date / time / operator

Reconciliation and review

Student: _____ Partner: _____ Date: _____

Opening Medium A balance (mL): _____ How verified: _____

Record each draw once. Include rejected and replacement draws and separately measured loss.

Record units and actual observations. Use N/A for measurements not performed.

Medium A use

Draw or loss ID	Tube / reason	Volume used (mL)	Date / time / operator

Label reconciliation

Issued	Applied	Spoiled / destroyed	Returned	Difference

Material reconciliation

Total use (mL)	Expected closing (mL)	Observed closing (mL)	Variance (mL)

Yield and review record

Student: _____ Partner: _____ Date: _____

Observed closing balance measurement method: _____

Inventory entry reference: _____ Update date/time/operator: _____

Accepted tubes: _____ Rejected tubes: _____ Replacement tubes: _____

Yield (%): _____ Operator review/signature/date: _____

Independent reviewer/date: _____ Review outcome: _____

Batch disposition / authorized person / date: _____

Record units and actual observations. Use N/A for measurements not performed.

Discrepancies and supporting records

Reference ID	Issue / affected material	Action / hold status	Reviewer / outcome

Calculations and observations

Expected closing balance = opening balance – total documented use. Variance = observed closing balance – expected closing balance. Labels issued = applied + spoiled or destroyed + returned. Yield (%) = accepted tubes ÷ 4 × 100.

Observations deviations and corrective actions

CONCLUSIONS

1. How does the executed BPR show whether the batch followed the MBR? Cite two actual entries.
2. Do the label and material reconciliations balance? Show the calculations and explain any difference.
3. Which tubes met the stated acceptance criteria, and what was the final yield? Explain any rejected or replacement units.
4. What additional information would the independent reviewer need to resolve any open discrepancy before disposition?

5: Cryopreserving Mammalian Cells Lab

Learning Objectives

- Explain how freezing medium and the cooling method can improve cell survival after thawing.
- Assess the culture's identity, growth, proportion of living cells, and total cell number before freezing.
- Calculate the freezing-medium volume and number of vials from the target for the assigned cells.
- Prepare, label, freeze, and record vials using the approved procedure for the assigned cells.

BACKGROUND

Cryopreservation (freezing cells for later use) allows a laboratory to store a dependable supply of cells. A **cell bank** (a collection of stored, identified cell vials) reduces the need to keep every culture growing continuously. Cells must survive both the freezing and the later thaw. Their condition before freezing matters: cultures that are unhealthy, contaminated, or too old may recover poorly even when frozen correctly. Before banking, check the assigned requirements for identity, **passage number** (the number of times a culture has been transferred to fresh vessels), contamination status, and **viability** (the percentage of cells that are alive). Record the results rather than assuming the culture is suitable.

As water freezes, ice and changes in the amount of dissolved substances can damage cell membranes. A **cryoprotectant** (a substance added to reduce freezing injury) helps protect the cells. **Dimethyl sulfoxide (DMSO)** (a common cryoprotectant used for mammalian cells) is present in many freezing media. Many procedures use about 5–10% DMSO, but the correct concentration and formulation depend on the cell type. Some commercial media have different or undisclosed formulations, and some contain no DMSO. Use only the medium specified for your assigned cells. Do not add DMSO to a ready-made medium unless the approved procedure directs you to do so. Because exposure to cryoprotectant before cooling can also stress cells, prepare the vials and cooling equipment before mixing cells with the medium.

The **cooling rate** (the speed at which the temperature falls) affects cell survival. Cooling too quickly can increase harmful ice formation; cooling too slowly can expose cells to damaging changes in dissolved substances. Many mammalian-cell procedures aim for about 1 °C per minute, but the assigned procedure controls the required method. A **controlled-rate freezing container** (a device designed to slow cooling at a predictable rate) or a programmable freezer provides this control. Some procedures use a –80 °C freezer as an intermediate step before transfer to long-term storage, often in the **vapor phase of liquid nitrogen** (the very cold gas above the liquid). Follow the specified temperature and transfer schedule. Placing vials directly on a –80 °C shelf does not establish a controlled cooling rate.

A **cryovial** (a vial made for freezing and storing biological samples) must be approved for the intended storage system and filled within its manufacturer's limit. Before filling, label each vial with enough information to identify its contents and connect it to the **cell-bank record** (the document that tracks preparation and storage of each vial). The record includes the source culture, cell count, freezing medium, time of cryoprotectant addition, cooling method, and storage position. The laboratory may later test a vial after thawing to see how well the cells recover. Your assigned procedure supplies the cell-specific target number of living cells per vial, fill volume, centrifuge settings, and timing; do not substitute values from another cell line.

Calculation example: If a suspension contains 12×10^6 living cells and the target is 3×10^6 living cells in each 1 mL vial, you can fill four vials. Prepare 4 mL of final cell suspension at 3×10^6 living cells/mL. If processing reduces the available cell count, document the actual result and follow the instructor's directions for changing the vial count.

MATERIALS

- Healthy assigned cell culture and its cryopreservation procedure
- Appropriate cell harvest reagents and complete medium
- **Class II biosafety cabinet** (a ventilated work enclosure that helps protect cultures and workers) and required personal protective equipment
- **Hemocytometer** (a counting chamber) or cell counter; **trypan blue** (a dye used to distinguish living from dead cells); microscope
- Sterile pipettes, tips, centrifuge tubes, and centrifuge when required
- Specified cryopreservation medium with verified identity, lot, and expiration

- Sterile cryovials approved for the intended storage system; cryogenic labels or marker
- Validated controlled-rate freezing container or programmable freezer
- Intermediate freezer and qualified long-term storage system specified by the procedure
- **Cryogenic PPE** (protective equipment for working around extremely cold materials), vial rack, storage map, and cell-bank record

METHODS

1. Read the procedure for your assigned cells. Record the allowed passage range, minimum viability, freezing medium, target living cells per vial, fill volume, centrifuge settings, cooling method, and storage destination. Ask the instructor about any missing values before beginning.
2. Inspect the culture and record cell identity, source vessel, passage number, morphology or suspension appearance, growth state, and any suspected contamination. Do not bank a suspect culture until it has been assessed under the laboratory procedure.
3. Prepare the biosafety cabinet and the required medium and supplies. Check that the freezing container and intermediate freezer are available and that long-term storage space has been assigned. Plan the batch so filled vials do not wait unnecessarily after exposure to cryoprotectant.
4. Harvest **adherent cells** (cells attached to a culture surface) using their approved detachment procedure, or collect **suspension cells** (cells that grow dispersed in liquid) using their assigned method. If **centrifugation** (spinning a sample to separate cells from liquid) is required, use the specified **relative centrifugal force** (the force applied to the sample, expressed as multiples of gravity) and time rather than a generic $600 \times g$ setting. Record the actual settings.
5. Mix the cell suspension gently and take a representative sample. Count living and dead cells using the assigned method. Record living cells/mL, percent viability, and total suspension volume. Calculate total living cells = living cells/mL $\times$ suspension volume in mL.
6. Divide the total living cells by the target living cells per vial to find the possible number of vials. Calculate the final suspension volume = planned vials $\times$ fill volume per vial. Calculate target living cells/mL = target living cells per vial $\div$ fill volume per vial. Record your calculations and any approved adjustment for cell loss. Obtain a calculation check if required.
7. Label each empty cryovial before filling with cell identity, unique bank or batch ID, passage number, vial number, freeze date, and other identifiers required by the laboratory. Put full details and operator identities in the bank record; do not rely on a date and initials alone to distinguish vials.
8. If the procedure calls for a **cell pellet** (cells collected at the bottom of a tube after centrifugation), remove the **supernatant** (liquid above the pellet) as directed. Gently suspend the cells in the specified volume of cryopreservation medium at the required temperature. Mix sufficiently for an even cell distribution without vigorous pipetting. Record the time cryoprotectant was added.
9. Dispense the specified volume into each pre-labeled cryovial, using the cryovial manufacturer's fill limit and leaving appropriate headspace. Cap securely. Record actual vial count and fill volume, reconcile filled, rejected, and unused vials, and cross-check the labels against the bank record.
10. Place the vials in the validated freezing container or programmable freezer and start the approved cooling program promptly. Record container or freezer ID, cooling program, placement time, and any interruption. Follow the device instructions for a passive container placed at -80°C .
11. At the specified time, use cryogenic PPE and the laboratory transfer procedure to move vials to the designated long-term storage location. Record transfer time, storage system, rack, box, and position for every vial. Do not treat -80°C intermediate storage as a permanent location unless the cell-specific procedure explicitly permits it.
12. Complete the cell-bank record, clean the work area, and schedule any required **post-thaw quality check** (a test of cells after a stored vial is thawed). Do not refreeze a thawed vial unless a specific validated procedure authorizes that action.

EXERCISE Cryopreserving Mammalian Cells NAME_____

EXPECTATIONS

1. Predict how a culture with many cells but low viability might perform after thawing. Explain your prediction.

2. Predict how a long delay after adding freezing medium or an incorrect cooling rate might affect survival after thawing.

RESULTS

Enter your observations and measurements below. Attach your cell-count worksheet if you used one. Show all vial and volume calculations. Complete the post-thaw fields only if a vial is tested.

Culture and freezing plan

Student: _____ Partner: _____ Date: _____

Cell identity/source: _____ Source vessel: _____ Passage: _____

Culture appearance/growth state: _____

Assigned passage range: _____ Minimum viability (%): _____

Freezing medium/lot: _____ Required temperature: _____

Target living cells/vial: _____ Planned fill volume (mL): _____

Centrifuge ID / actual force / time / temperature, if used: _____

Record units and actual observations. Use N/A for measurements not performed.

Cell count and vial planning

Living cells/mL	Viability (%)	Suspension volume (mL)	Total living cells

Preparation and cooling record

Event	Actual date / time	Device / program / details	Operator
Cryoprotectant added			
Vials filled			
Cooling started			
Transfer to long-term storage			

Cryovial inventory and quality checks

Student: _____ Partner: _____ Date: _____

Bank or batch ID: _____ Planned vials: _____ Actual filled vials: _____

Rejected vials/reason: _____ Unused vials: _____

Cooling program / container ID: _____ Intermediate freezer ID: _____

Storage system ID: _____ Required transfer interval: _____

Record units and actual observations. Use N/A for measurements not performed.

Vial storage record

Vial ID	Fill volume (mL)	Living cells/vial	Rack / box / position	Transfer date / time

Post-thaw check if performed

Vial ID / thaw date	Viability (%)	Growth / appearance	Check time / assay	Outcome

Calculations and observations

Total living cells = living cells/mL × suspension volume. Possible vials = total living cells ÷ target living cells per vial. Final suspension volume = planned vials × fill volume. Target living cells/mL = target living cells per vial ÷ fill volume.

Observations deviations and corrective actions

CONCLUSIONS

1. Did your culture meet the assigned requirements for freezing? Use its appearance, growth state, passage number, and viability to support your answer.
2. Show your calculation for the number of vials and living cells per vial. If the actual vial count differed from your plan, explain why.
3. Which recorded details would help another student identify and locate one of your vials and explain how it was frozen?
4. If the thawed cells show poor survival, identify two possible causes using your records. What information would you check to investigate each cause?

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6: Media Changes and Cell Counting Lab

Learning Objectives

- Explain how medium renewal differs for cells that attach to a vessel and cells that grow in the liquid.
- Renew medium without disturbing an attached cell layer or discarding cells growing in the liquid.
- Count living and dead cells to calculate living cells per milliliter and the percentage that are alive.
- Record cell observations, medium lots, counts, calculations, and any deviations.

BACKGROUND

Culture medium (the nutrient-containing liquid in which cells grow) supplies nutrients and helps maintain suitable chemical conditions around cells. Cells consume nutrients and release waste, so the medium must be renewed according to the needs of that cell type. Before changing it, look at the color of the medium and at the cells' shape, growth, and density. A **medium change** (removing or adding medium to refresh the culture) is different from **passaging** (moving a portion of a culture into a new vessel to continue growth). A medium change does not automatically reduce the number of cells. Your observations help determine whether feeding, dilution, passaging, or investigation is appropriate.

Adherent cells (cells that grow attached to a culture surface) remain on the bottom of their vessel while spent medium is removed. **Normal human dermal fibroblasts (NHDFs)** (cells derived from human skin connective tissue) are an example. For these cultures, remove old medium gently without touching the attached **monolayer** (the layer of cells on the growth surface), then add fresh medium along the vessel wall. **Confluence** (the proportion of growth surface covered by cells) helps show whether the culture is approaching the point at which it may need passaging. Do not rinse with **phosphate-buffered saline (PBS)** (a salt solution used to rinse cells) unless the assigned procedure calls for it, because an unnecessary rinse can dislodge cells.

Suspension cells (cells that grow dispersed in liquid medium) require different handling. **THP-1 cells** (a human cell line that normally grows in suspension) are an example. Discarding their medium would also discard the cells. The assigned procedure may direct you to add fresh medium, dilute and transfer some culture, or collect cells for complete medium replacement. **Cell density** (the number of cells in a specified volume, often cells/mL) helps you decide how much medium to add. The acceptable density range and renewal method depend on the specific cells and culture conditions.

A **hemocytometer** (a counting slide with a grid of known dimensions) allows you to estimate cell concentration from a small sample. **Trypan blue** (a dye that enters cells with damaged membranes) usually stains dead cells blue while living cells remain clear. Mixing equal volumes of cell suspension and dye produces a **twofold dilution** (one part sample in two parts final mixture). Count four large corner squares in a standard chamber: $\text{living cells/mL} = (\text{total clear cells} \div 4) \times 10,000 \times 2$. Calculate total cells/mL using clear plus blue cells. **Percent viability** (the percentage of counted cells that are alive) = $\text{clear cells} \div (\text{clear cells} + \text{blue cells}) \times 100$. For example, 200 clear and 20 blue cells across four squares give 1.0×10^6 living cells/mL and 90.9% viability. The 10,000 factor applies to large 1 mm² squares in a standard chamber 0.1 mm deep; use a different factor if your device specifies one.

A count describes the concentration in the suspension you actually sampled. Multiply living cells/mL by the full suspension volume in mL to estimate the total number of living cells. If you deliberately dilute the counting sample again before loading it, include that additional dilution in the calculation. For example, an extra one-to-five sample dilution multiplies the calculated concentration by five. Do not add a dilution factor simply because you earlier resuspended the entire culture in a larger volume; that volume is already represented by the sample's measured concentration. Record the count in each square so you can spot uneven loading or cell clumps.

MATERIALS

- Assigned adherent and/or suspension cultures with cell-specific handling instructions
- Appropriate complete growth medium and culture vessels
- **Class II biosafety cabinet** (a ventilated enclosure that helps protect cultures and workers) and required personal protective equipment
- Sterile **serological pipettes** (graduated pipettes for measuring milliliter volumes) or sterile tips and pipette aid
- Disinfectant, wipes, and suitable liquid waste container

- Hemocytometer and correct coverslip
- Microscope and 0.4% trypan blue
- **Micropipette** and tips suitable for 10–20 μL transfers
- Microcentrifuge tubes and cell culture record
- Centrifuge and tubes only if required by the assigned suspension-culture procedure

METHODS

Before Handling Cultures

1. Read the instructions for the assigned cells. Record their medium, feeding or subculture limits, vessel, culture volume, and incubation conditions. Determine whether the task is a medium change, a dilution, or a separate passaging procedure.
2. Examine each culture under the microscope before opening it. Record date, cell identity, passage number when known, morphology, estimated confluence for adherent cultures or density for suspension cultures, medium appearance, and any sign of contamination. Report suspected contamination before proceeding.
3. Prepare the biosafety cabinet according to the laboratory procedure. Bring in the correctly labeled medium, sterile supplies, and waste container. Use medium at the temperature specified for the assigned cells; avoid unnecessarily prolonged warming.

Adherent Culture Medium Change

4. Keep the flask level and inspect the cell layer. Open it in the cabinet. Gently remove spent medium by **aspiration** (drawing liquid out by suction) or pipetting without contacting or flooding the monolayer. Do not pour the contents into a waste bottle.
5. Do not rinse with PBS unless the assigned procedure specifically requires it. Slowly add the prescribed volume of fresh complete medium along the vessel wall. Confirm the cell layer is covered and the cap is set correctly.
6. Return the vessel promptly to its specified incubator. Record the medium name, lot, volume added, time, operator, and observations after feeding. If the culture is approaching its passaging limit, notify the instructor and use the separate passaging procedure.

Suspension Culture Medium Renewal

7. Mix the closed culture gently as directed so the cells are evenly distributed. Take an aseptic sample for cell counting before deciding the renewal volume. Do not discard the spent medium because it contains the cells.
8. Calculate the volume of existing culture and fresh medium needed to reach the target density or renewal ratio in the cell-specific procedure. Add medium or transfer an appropriate **aliquot** (a measured portion of a sample) to a labeled vessel. If that procedure requires complete medium replacement, collect cells and use its specified centrifugation force and time before resuspending; record those settings.
9. Mix gently and return the vessel to the specified incubator. Do not assume a shaking flask or spinner is appropriate for every THP-1 culture. Record the starting and final volumes, measured density, medium lot, time, operator, and vessel ID.

Hemocytometer and Trypan Blue Count

10. Mix the cell suspension gently immediately before sampling. If counting adherent cells, first prepare a single-cell suspension using the separate approved detachment procedure. Label a small tube with sample identity and any planned dilution.
11. Mix 10 μL of cell suspension with 10 μL of 0.4% trypan blue. Record the twofold dye dilution and count promptly according to the dye manufacturer's timing instructions.
12. Clean the hemocytometer and place its proper coverslip. Load a small amount of mixture at the edge of one chamber so **capillary action** (liquid moving into a narrow space) fills the grid without bubbles or overflow. Do not pipette directly onto the grid. Load a second chamber if the course procedure requires repeated counts.
13. Under the microscope, count clear and blue cells separately in four large corner squares. Apply one consistent boundary rule, such as including cells touching the top and left lines and excluding those touching the bottom and right lines. Record each square separately; recount if cells are unevenly distributed or too crowded.

14. Add the clear and blue counts across four squares. Divide each total by four to find the average per square. Calculate living cells/mL, total cells/mL, and percent viability. Include the twofold dye dilution and any other dilution you actually made to the counting sample. Multiply living cells/mL by the culture volume if you need the total living cells. Show your work and units.

15. Dispose of trypan blue and cell-containing waste as directed, clean the hemocytometer according to its care procedure, disinfect the work area, and complete the culture record.

EXERCISE Media Changes and Cell Counting NAME_____

EXPECTATIONS

1. Predict how the handling of spent medium will differ between an adherent NHDF culture and a THP-1 suspension culture. What would happen if the suspension medium were poured away?

2. If the same culture is counted before and after adding fresh medium without losing cells, how should cells/mL and total viable cells change? Explain your prediction.

RESULTS

Record observations and numbers below. Show the calculation for living cells/mL, total cells/mL, percent viability, and total living cells when needed. Describe any unusual observation separately from your interpretation.

Culture and medium renewal record

Student: _____ Partner: _____ Date: _____

Cell identity: _____ Passage: _____ Culture type: _____

Assigned medium/feeding limits: _____

Incubator conditions: _____ Medium temperature: _____

Centrifuge ID / actual force / time, if used: _____

Record units and actual observations. Use N/A for measurements not performed.

Culture inspection

Vessel ID	Date / time	Confluence or density and units	Cell / medium appearance	Action selected

Medium renewal

Vessel ID	Medium / lot	Starting culture (mL)	Culture retained (mL)	Fresh medium (mL)	Final volume (mL)

Hemocytometer count worksheet

Student: _____ Partner: _____ Date: _____

Sample/vessel ID: _____ Count date/time: _____ Operator: _____

Cell suspension volume mixed with dye (μL): _____ Dye volume (μL): _____

Dye dilution factor: _____ Other sample dilution factor: _____ Total factor: _____

Boundary rule used: _____ Loading or distribution problems: _____

Record units and actual observations. Use N/A for measurements not performed.

Raw counts

Chamber / repeat	Square	Clear living cells	Blue dead cells
1 / 1			
1 / 2			
1 / 3			
1 / 4			
2 / 1 if used			
2 / 2 if used			
2 / 3 if used			
2 / 4 if used			

Calculated count results

Sample / repeat	Mean living cells/square	Living cells/mL	Total cells/mL	Viability (%)	Total living cells

Calculations and observations

For each chamber, divide the counts by the number of squares counted. Living cells/mL = mean living count per large square $\times 10,000 \times$ total dilution factor. Total cells/mL = mean living plus dead count $\times 10,000 \times$ total dilution factor. Viability (%) = living count $\div$ (living + dead count) $\times 100$. Total living cells = living cells/mL $\times$ culture volume in mL.

Observations deviations and corrective actions

CONCLUSIONS

1. Was the medium-change method appropriate for the growth pattern of your cells? Use specific observations and steps to support your answer.
2. Show how you calculated living cells/mL and percent viability. Explain each dilution factor you used.
3. How did the observed cell density and viability influence your decision to feed, dilute, passage, or investigate the culture?
4. Identify one possible source of error in the hemocytometer count and explain how it would change the reported concentration or viability.

7: Thawing and Establishing a Mammalian Cell Culture

Learning Objectives

- By the end of this exercise, students should be able to:
- Distinguish **primary cells** from **continuous cell lines** and adherent from suspension cultures.
- Explain why cryopreserved cells are thawed promptly and handled gently during recovery.
- Prepare a biosafety cabinet and culture vessel using the instructions for the assigned cell type.
- Document the source vial, medium, recovery method, cell observations, and any departures from the procedure.

BACKGROUND

Cell culture and growth patterns

Cell culture is the maintenance and growth of cells outside the body under controlled conditions. In this exercise, you will thaw an assigned mammalian cell sample, establish a culture, and observe its early recovery. This procedure introduces the skills you will later apply when working with **THP-1** and **NHDF** cells.

Primary cells are cells collected directly from tissue. They often keep many features of the original tissue, but usually divide only a limited number of times. A **cell line** is a population of cells maintained through repeated growth and transfer. Continuous cell lines can divide over many passages under suitable conditions. The source and history of a culture can affect its growth and response to an experiment.

Adherent cells attach to a suitable surface to grow. **Suspension cells** grow in the liquid medium without needing to attach. These growth patterns affect vessel choice, feeding, counting, and recovery checks. This exercise focuses on establishing one of these two culture types.

Culture conditions and aseptic handling

Culture medium is a liquid mixture that supplies nutrients and supports a suitable chemical environment. **Complete medium** contains the base medium and all supplements required by the assigned cells. Different cells may need different nutrients and growth factors. Use the handling sheet for the actual product and lot rather than assuming that all mammalian cells use the same medium.

A **cell culture incubator** maintains the temperature and gas conditions needed for growth. Many mammalian cultures use 37 °C, but temperature, carbon dioxide level, and other settings must match the medium and cell-specific instructions. Prepare the medium, vessel, and incubator before retrieving frozen cells.

Aseptic technique is a set of practices that helps prevent unwanted microorganisms or other cells from entering a culture. This unwanted material is called **contamination**. A **Class II biosafety cabinet** uses controlled airflow and filtration to help protect the culture, the worker, and the surrounding area. It does not make contaminated items sterile. Use sterile supplies, disinfect surfaces as directed, and keep containers open only as long as needed. Handle human-derived cells under the laboratory's approved biosafety practices.

Freezing and thawing

Cryopreservation is the storage of cells at very low temperatures to greatly slow cell activity. A **cryoprotectant** is a substance that helps reduce freezing damage. **Dimethyl sulfoxide**, or **DMSO**, is commonly used for this purpose. Freezing and thawing can still damage the **cell membrane**, the barrier that controls movement into and out of a cell. Some cells may die even when the procedure is followed correctly.

A prompt thaw helps limit damage as ice melts. Gentle transfer into the correct medium reduces stress from sudden changes in the cells' surroundings. Prolonged exposure to DMSO after warming may harm cells. Follow the handling sheet for the thawing endpoint, dilution, and whether the cryoprotectant should be removed.

Centrifugation uses spinning to collect cells into a **pellet** at the bottom of a tube. The **supernatant** is the liquid above the pellet. When directed, remove this liquid and **resuspend** the cells by gently mixing them into fresh medium. Some cell products should be seeded without immediate centrifugation. Use the specified **relative centrifugal force**, expressed as $\times g$, and time when centrifugation is required. These settings describe the force applied to the cells and should not be replaced with an assumed rotor speed.

Checking recovery

Viability describes how many cells in a sample are alive. A **viability dye** helps distinguish living cells from dead cells. For example, **trypan blue** enters cells with damaged membranes and makes them appear blue. A **hemocytometer** is a chamber with a marked grid used to count cells in a known volume. An approved cell counter may also be used.

Percentage viability = living cells counted $\div$ total cells counted $\times 100$.

Cell density is the number of cells in a given volume or surface area. **Seeding density** is the density used to start a culture. For suspension cells, report **viable cell concentration** as living cells per milliliter. For adherent cells, seeding density is often reported as cells per square centimeter of growth surface. Choose the medium volume and vessel using the recommended range.

Morphology means cell shape and appearance. Recovering adherent cells usually attach and spread, while suspension cells remain in the medium. **Confluence** is the percentage of a surface covered by adherent cells. It does not describe a suspension culture. A cell that excludes a viability dye may still fail to grow, so evaluate recovery through follow-up observations as well as the initial count.

Culture records

Traceability is the ability to connect a culture to its source and handling history. A **lot number** identifies a supplied batch. A **passage number** records how many times a culture has been transferred, using the laboratory's counting system. Record these identifiers, the operator, thaw date, medium and supplement lots, vessel, seeding conditions, and observations.

A **deviation** is a departure from the approved procedure. Record deviations and report possible contamination or poor recovery to the instructor. Unexpected cloudiness, particles, or cell shapes may signal a problem, but appearance alone cannot rule out contamination. Clear records help explain the outcome and support later experiments.

MATERIALS

- Assigned cryopreserved cell vial and its supplier or laboratory handling sheet
- Class II biosafety cabinet and required personal protective equipment
- Appropriate complete growth medium and pre-labeled culture vessel
- Sterile pipettes, tips, and pipette aid appropriate to the transfer volume
- 37 °C water bath or approved dry-thaw device
- Disinfectant and clean wipes specified by the laboratory
- Sterile centrifuge tube and balanced centrifuge
- Microscope and cell counter or hemocytometer, if required by the procedure
- Biohazard waste container and laboratory culture record

METHODS

1. Read the assigned vial's handling sheet. Record the cell identity, vial or lot number, specified medium, vessel type, thaw method, DMSO-removal instructions, seeding density, and incubation conditions. Have the instructor resolve any missing or conflicting instructions before beginning.
2. Prepare and verify the labeled culture vessel and the required volume of appropriate medium. Record medium and supplement lot numbers. Set up the biosafety cabinet according to the laboratory procedure and bring in only the needed supplies.
3. Put on the protective equipment required for cryogenic storage. Retrieve one vial using the laboratory's cryogen-handling procedure. If a vial may have been immersed in liquid nitrogen or appears damaged, do not warm it; isolate it and notify the instructor because trapped liquid nitrogen can cause pressure buildup.
4. Thaw the vial promptly by gently moving it in the 37 °C water bath, keeping the cap and seal above the water, or use the approved dry-thaw device. Follow the handling sheet for the endpoint. Do not leave a thawed vial warming in the bath.

5. Wipe the exterior of the vial with the approved disinfectant before bringing it into the biosafety cabinet. Allow the disinfectant to act for the required time. Open the vial only inside the cabinet.
6. Transfer the cell suspension gently into the specified complete medium. Follow the **assigned cell-specific procedure** for dilution and for removal or retention of cryoprotectant. If the instructions require a centrifugation step, use the specified relative centrifugal force and time, balance the rotor, remove supernatant as directed, and gently resuspend the cells. If the instructions say to seed without centrifugation, do so.
7. Count cells and assess viability if required. Calculate and record the volume needed to achieve the specified viable-cell seeding density. Transfer the cells to the appropriate vessel: a suitable attachment surface for adherent cells or the specified vessel for suspension cells.
8. Distribute the suspension gently as directed for the vessel type, avoiding liquid in the cap or filter area. Place the vessel in the incubator at the conditions specified on the handling sheet. Record the seeding time, vessel ID, medium volume, cell density when known, and incubator location.
9. Inspect the culture at the time specified by the instructor or handling sheet. For adherent cells, record attachment and morphology; for suspension cells, record appearance, density when measured, and evidence of clumping. Note possible contamination or other unexpected changes and report them promptly.
10. Disinfect the work area, dispose of waste under the laboratory procedure, remove PPE appropriately, and complete the culture record, including any deviations and follow-up observations.

EXERCISE Thawing and Establishing a Mammalian Cell Culture **NAME** _____

EXPECTATIONS

1. What growth pattern do you expect for your assigned cells, and which observations would show early recovery?
2. How might prolonged warming or an inappropriate centrifugation step affect recovery, and which measurements could help you evaluate that effect?

RESULTS

Record the cell identity, vial lot, and medium and supplement lots. Include thawing and transfer times, the recovery method, and any centrifugation settings. Report the viable cell count and seeding density when measured. Record the vessel, incubation conditions, and initial and follow-up observations. Include calculations and deviations. Keep your observations separate from your explanations.

Student: _____ Partner: _____ Date: _____

Cell identity: _____ Source or supplier: _____

Vial or lot number: _____ Passage number if known: _____

Growth pattern (adherent or suspension): _____

Handling sheet or procedure reference: _____

Medium and vessel record

Medium or supplement	Lot number	Amount and units
Complete medium		

Supplement if added		
Supplement if added		

Culture vessel type and ID: _____ Growth surface area if needed (cm²): _____

Thawing and recovery record

Event	Actual time	Method settings or observations
Thawing started		
Thawing endpoint reached		
Cells transferred into medium		
Cells seeded		

Cryoprotectant retained or removed and method: _____

Centrifugation if performed: force (× g) _____ Time (min) _____ Temperature (°C) _____

Record N/A for steps or measurements not performed.

Cell count and seeding data if measured

Measurement	Value and units
Counting method and sample ID	
Living cells counted	
Dead cells counted	
Counting sample dilution factor	
Living cell concentration (cells/mL)	

Viability (%)	
Cell suspension volume (mL)	
Target seeding density (cells/mL or cells/cm ²)	
Cell suspension volume transferred (mL)	
Actual living cells seeded	
Actual seeding density (cells/mL or cells/cm ²)	
Final medium volume in culture vessel (mL)	

Seeding calculations if needed

Show the calculation used to reach the assigned seeding density. Include units.

Incubator ID and location: _____ Temperature (°C): _____ CO₂ (%): _____

Other incubation conditions required by the handling sheet: _____

Initial and follow up culture observations

Date and time	Attachment or suspension appearance	Confluence or density if measured	Clumping medium changes or other observations

For adherent cells, record attachment and confluence when measured. For suspension cells, record appearance, clumping, and density when measured.

Deviations reported concerns and actions taken

CONCLUSIONS

1. Did the observed attachment or suspension behavior match the expected growth pattern for the assigned cells? Use your observations to explain.
2. What evidence suggests that the cells recovered successfully, and what additional observation or measurement would strengthen your conclusion?
3. If viability or growth was lower than expected, identify two plausible causes related to thawing or seeding and explain how you would investigate each one.
4. How did the cell-specific instructions change your procedure, particularly the choice to remove or retain the cryoprotectant and whether to centrifuge?

Attributions:

ATCC. Animal Cell Culture Guide. <https://www.atcc.org/-/media/resourc...ture-guide.pdf>

ATCC. Primary Cell Culture Guide. <https://www.atcc.org/en/resources/cu...-culture-guide>

8: Thawing THP-1 and NHDF Cells

Learning Objectives

- Apply the introductory thawing procedure to **THP-1** and **NHDF** cells using their specific handling instructions.
- Plan THP-1 **viable cell concentration** and NHDF **seeding density** using the correct units.
- Compare recovery using observations suited to each cell type.

BACKGROUND

Applying the introductory exercise

This exercise follows Thawing and Establishing a Mammalian **Cell Culture**. You will apply the same preparation, aseptic handling, thawing, and documentation skills to two human cell types. The focus is to select the correct conditions for each culture and compare evidence of recovery. Review the introductory exercise for the general explanations of **cryopreservation**, **DMSO**, **viability**, and cell counting.

THP-1 cells

THP-1 is a continuous human **cell line** originally obtained from a patient with **acute monocytic leukemia**, a cancer of blood-forming cells. These cells are commonly used to study immune responses. Under the routine conditions used here, they grow in suspension and usually appear round. Treatments that cause **differentiation**, or a change toward a more specialized cell state, can alter their appearance and attachment. This exercise examines recovery without such treatment.

For THP-1, culture recovery is assessed using viable cell concentration, percentage viability, **morphology**, and growth over time. A flask with few visible cells may simply contain too much medium for the number of surviving cells. Plan the starting concentration using living cells rather than the total count. Follow the lot-specific recommendation for medium volume and flask size.

Starting medium volume in mL = total viable cells available ÷ target viable cells per mL.

Use this calculation when all available viable cells will be seeded. If only part of the sample is used, calculate the viable cells in that portion first. Mix the suspension gently before taking a counting sample so it represents the culture. Record clumps because they can make counts less reliable. **Confluence** is not used to assess THP-1 suspension growth.

NHDF cells

Normal human dermal fibroblasts, or NHDF cells, are **primary cells** from the **dermis**, the layer of skin below the outer surface. **Fibroblasts** help maintain connective tissue and support wound repair. NHDF cells are adherent and require a suitable tissue culture surface. They may look round immediately after thawing, then attach, spread, and develop an elongated, spindle-like shape during recovery.

NHDF seeding is planned using the recommended number of cells per square centimeter of growth surface. Flask growth area and medium volume are separate measurements. Increasing the medium volume does not increase the area available for attachment.

Cells needed = target cells per cm² × flask growth area in cm².

Use the supplier's stated basis for this calculation, including whether the recommendation refers to viable cells. Select a flask area that matches the cells available. Gently distribute cells across the surface and avoid unnecessary movement while they attach. Evaluate recovery by observing attachment, spreading, morphology, confluence, and later growth. A single early observation of round cells does not prove that the culture has failed.

Because NHDF cells have a limited ability to divide, their passage history matters when planning later experiments. Record the supplied passage information and use the laboratory's approved tracking system. Growth and recovery may also vary with the donor and lot.

Choosing conditions for each cell type

Prepare separate complete media and clearly labeled vessels for THP-1 and NHDF. Verify the exact product and lot instructions for each. The ATCC THP-1 procedure includes **centrifugation** to remove freezing medium. NHDF recovery instructions can differ by supplier and product, including whether immediate centrifugation is recommended. Follow the instructions supplied with the actual vials.

Use separate sterile transfer supplies to prevent mixing the two cultures. Complete the thawing and seeding of one vial before retrieving the next. Record any differences in the recovery procedures, including **cryoprotectant** removal, vessel selection, starting density, and the timing of medium changes.

Comparing recovery

At each scheduled check, compare the cultures using measures suited to their growth patterns. For THP-1, an increase in viable cell concentration over time supports recovery. For NHDF, attachment and spreading followed by growth across the surface support recovery. Good initial viability alone is not enough to show that either culture is ready for an experiment.

If recovery is poor, review the records for that cell type. Consider its medium, thawing and transfer times, cryoprotectant-removal method, starting density, and incubation conditions. Report unusual findings to the instructor before changing the procedure. Use the laboratory's recovery and **contamination** criteria to decide when each culture can be used.

MATERIALS

- Frozen vials of THP-1 and NHDF cells
- Separate, appropriately supplemented complete media for THP-1 and NHDF
- Labeled tissue culture flasks of appropriate size
- 37 °C water bath (not a hot water bath)
- Certified biosafety cabinet and appropriate personal protective equipment
- 70% ethanol or laboratory-approved vial disinfectant
- Sterile transfer pipettes and pipette tips
- Sterile centrifuge tubes and centrifuge, if the product instructions require cryoprotectant removal
- Cell counter or **hemocytometer** and **viability dye**
- **Cell culture incubator**

METHODS

1. Check each vial label, cell identity, passage or lot information, and product instructions. Label separate flasks for THP-1 and NHDF. Prepare their correct complete media and bring the flasks or media to the appropriate culture temperature.
2. Plan the THP-1 starting viable cell concentration and NHDF seeding density using the supplier's ranges. Choose the appropriate flask growth area and medium volume for each culture. Record your calculations.
3. Follow the cryogenic handling procedure from the introductory exercise. Retrieve one vial and thaw it promptly in a 37 °C water bath with the cap and seal above the water. Follow its handling sheet for the endpoint; do not continue warming a thawed vial.
4. Disinfect the vial exterior for the required contact time and transfer it into the biosafety cabinet. Open it and gently mix the contents using **aseptic technique**.
5. Transfer the cells into their correct **complete medium**. Follow their product instructions for dilution and cryoprotectant removal. If centrifugation is required, use the specified force and time, balance the rotor, and gently **resuspend the pellet**.
6. For THP-1, mix gently, determine viable cell concentration if possible, and adjust the medium volume to the recommended starting density. Transfer to the labeled suspension culture flask.
7. For NHDF, seed the cells into a suitable attachment flask at the recommended density. Move the flask gently front-to-back and side-to-side to distribute the cells. After completing seeding for the first cell type, repeat steps 3–7 for the other vial using separate labeled supplies.
8. Place each flask in the incubator under its specified conditions. Avoid disturbing NHDF cells during attachment. At the scheduled checks, record THP-1 viable cell concentration, viability, and morphology; record NHDF attachment, spreading, morphology, and confluence. Change or supplement medium as directed for each culture. Report unexpected findings.

9. Disinfect the work area, dispose of waste as directed, remove protective equipment, and complete a separate culture record for each cell type.

EXERCISE Thawing THP-1 and NHDF Cells NAME _____

EXPECTATIONS

1. How would the appearance of a successfully recovering NHDF culture differ from that of THP-1 during the first day after thawing?
2. What might happen to a THP-1 culture if the cells are diluted far below the recommended starting density?

RESULTS

Record the cell identity, vial lot, and medium and supplement lots. Include thawing and transfer times, the recovery method, and any centrifugation settings. Report the viable cell count and seeding density when measured. Record the vessel, incubation conditions, and initial and follow-up observations. Include calculations and deviations. Keep your observations separate from your explanations.

Student: _____ Partner: _____ Date: _____

Cell identity: _____ Source or supplier: _____

Vial or lot number: _____ Passage number if known: _____

Growth pattern (adherent or suspension): _____

Handling sheet or procedure reference: _____

Medium and vessel record

Medium or supplement	Lot number	Amount and units
Complete medium		
Supplement if added		
Supplement if added		

Culture vessel type and ID: _____ Growth surface area if needed (cm²): _____

Thawing and recovery record

Event	Actual time	Method settings or observations
Thawing started		
Thawing endpoint reached		

Event	Actual time	Method settings or observations
Cells transferred into medium		
Cells seeded		

Cryoprotectant retained or removed and method: _____

Centrifugation if performed: force ($\times g$) _____ Time (min) _____ Temperature ($^{\circ}C$) _____

Record N/A for steps or measurements not performed.

Cell count and seeding data if measured

Measurement	Value and units
Counting method and sample ID	
Living cells counted	
Dead cells counted	
Counting sample dilution factor	
Living cell concentration (cells/mL)	
Viability (%)	
Cell suspension volume (mL)	
Target seeding density (cells/mL or cells/cm ²)	
Cell suspension volume transferred (mL)	
Actual living cells seeded	

Measurement	Value and units
Actual seeding density (cells/mL or cells/cm ²)	
Final medium volume in culture vessel (mL)	

Seeding calculations if needed

Show the calculation used to reach the assigned seeding density. Include units.

Incubator ID and location: _____ Temperature (°C): _____ CO₂ (%): _____

Other incubation conditions required by the handling sheet: _____

Initial and follow up culture observations

Date and time	Attachment or suspension appearance	Confluence or density if measured	Clumping medium changes or other observations

For adherent cells, record attachment and confluence when measured. For suspension cells, record appearance, clumping, and density when measured.

Deviations reported concerns and actions taken

CONCLUSIONS

1. What observations indicate that THP-1 cells recovered after thawing?
2. What observations indicate that NHDF cells attached and recovered?
3. How could using the wrong medium or an unsuitable seeding density affect either culture?
4. If one culture recovered poorly, which recorded steps or measurements would you examine first, and why?

Attributions:

ATCC. THP-1 (TIB-202) product sheet: <https://www.atcc.org/api/pdf/product-sheet?id=TIB-202>

ATCC. Primary Dermal Fibroblast, Normal, Human, Adult (PCS-201-012): <https://www.atcc.org/products/pcs-201-012>

PromoCell. Fibroblasts product manual: <https://promocell.com/media/product-...al/C-12302.pdf>

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9: Passaging Suspension Cells

Learning Objectives

- Explain why cells growing in liquid culture must be transferred before they become too dense.
- Measure the number of living cells per milliliter and calculate the amount of culture needed for a new vessel.
- Transfer THP-1 cells into fresh medium using their assigned procedure.
- Record cell counts, medium and vessel identifiers, calculations, and observations before and after transfer.

BACKGROUND

THP-1 cells (a human cell line commonly used to study immune cell behavior) normally grow as a **suspension culture** (cells dispersed in liquid medium rather than attached to the vessel). **Passaging** (transferring some cells to a new vessel with fresh medium so they can continue growing) helps keep cells within a suitable density range. Because routine THP-1 cells are not attached, they usually do not need a detachment enzyme. As cells divide, the number of living cells per milliliter rises, nutrients are consumed, and wastes accumulate. If a culture becomes too dense, cell growth or health may change. Transferring a measured portion into fresh medium gives the new culture an appropriate starting density.

Count the cells before deciding how much culture to transfer. A **hemocytometer** (a counting slide with a grid of known dimensions) and **trypan blue** (a dye that stains many dead cells blue while living cells remain clear) let you estimate the number of living cells per milliliter. **Viability** (the percentage of counted cells that are living) is also useful because two cultures with the same total cell count may contain different numbers of living cells. Use the allowable **cell density** (the number of cells per milliliter) and target starting density specified in the assigned cell product sheet or laboratory procedure. These limits can differ among cell stocks and experiments. If the culture looks clumped or contaminated, report that observation before proceeding.

For a routine dilution, select the target concentration and final volume from the assigned procedure. The **dilution equation** (a relationship used to calculate how much original culture is needed to reach a target concentration) is $C_1V_1 = C_2V_2$. Here C_1 is the measured living-cell concentration in the original culture; V_1 is the volume of that culture to transfer; C_2 is the desired living-cell concentration in the new vessel; and V_2 is its final volume. Rearrange to find $V_1 = C_2V_2 \div C_1$. Fresh-medium volume = $V_2 - V_1$. For example, with $C_1 = 1.0 \times 10^6$ living cells/mL, $C_2 = 2.5 \times 10^5$ living cells/mL, and $V_2 = 20$ mL, transfer 5 mL of original culture and add 15 mL of fresh medium. The units cancel to give milliliters. Check that enough culture is available and that the new vessel can safely hold the final volume.

A routine dilution usually leaves the cells in their existing liquid and does not require a centrifuge. If a separate procedure calls for complete medium replacement, **centrifugation** (spinning a sample to collect cells) may be needed before adding fresh medium. Use only its specified force and time because processing can stress cells or cause cell loss. **PMA treatment** (exposure to phorbol 12-myristate 13-acetate, a chemical that can change THP-1 behavior) may cause cells to attach to the vessel; those cells need a different handling procedure. Record what you actually transfer and later compare the new culture's observed density with its calculated starting density.

MATERIALS

- THP-1 culture and its product sheet or laboratory **SOP** (standard operating procedure, an approved set of instructions)
- Appropriate complete growth medium and sterile receiving vessel
- **Class II biosafety cabinet** (a ventilated enclosure that helps protect cultures and workers) and required personal protective equipment
- Sterile **serological pipettes** (graduated pipettes for milliliter volumes), pipette aid, **micropipette** (a device for small microliter volumes), and sterile tips
- Hemocytometer, coverslip, microscope, 0.4% trypan blue, and sample tube
- Disinfectant, wipes, and appropriate liquid waste container
- Culture record and calculator
- Centrifuge and sterile tubes only if the assigned procedure requires them

METHODS

1. Review the assigned THP-1 product sheet or SOP for medium, allowed density range, target **reseeding density** (the intended living-cell concentration immediately after transfer), vessel, final volume, incubation conditions, and special handling. Label a new vessel with cell identity, date, passage number, and operator.
2. Inspect the culture under the microscope. Record appearance, clumping, medium condition, and possible contamination. Report suspected contamination before opening the vessel. Prepare the biosafety cabinet and bring in only required supplies.
3. Gently mix the closed culture to disperse settled cells without vigorous shaking. In the cabinet, remove a small **aseptic sample** (a sample collected while preventing contamination) sufficient for counting; do not withdraw one-tenth of the culture unless the procedure specifically requires it.
4. Count clear and blue cells using the hemocytometer and trypan blue method from the cell counting lab. Record each square's counts, the dye dilution and any additional sample dilution, living cells/mL, and percent viability. If the grid is too crowded or sparse to count reliably, prepare and document a suitable new sample.
5. Choose the target living cells/mL and final volume from the assigned instructions. Calculate $V_1 = C_2V_2 \div C_1$ and fresh-medium volume = $V_2 - V_1$. Show your units and ask a second student to check the calculations. Consult the instructor if you would need more old culture than is available or more final volume than the vessel allows.
6. Add the calculated fresh complete medium to the receiving vessel. Gently remix the source culture immediately before withdrawing V_1 . Transfer the calculated volume aseptically into the receiving vessel. Mix gently to distribute cells evenly; do not add cells to an empty vessel unless the cell-specific procedure directs it.
7. If complete medium replacement is explicitly required, follow that separate SOP for cell collection, centrifugation settings, **supernatant** (the liquid above collected cells) removal, and **resuspension** (mixing collected cells back into liquid). Record the settings and any cell loss. Do not substitute a generic $600 \times g$ spin.
8. Return the vessel to its specified incubator. Record source and receiving vessel IDs, cell passage number, actual volumes transferred and added, medium lot, date and time, identities of both students and the steps each performed. Note the calculated starting density in the new vessel.
9. At the next scheduled observation, remix and recount a sample as directed. Record cell density, viability, appearance, and any unexpected growth or contamination. Clean the cabinet and dispose of waste according to laboratory procedures.

EXERCISE Passaging Suspension Cells NAME_____

EXPECTATIONS

1. Predict what would happen to the new culture's starting density if you transferred twice the calculated old-culture volume but kept the same final volume. Explain your prediction.
2. Predict how using the same transfer volume from a culture with lower viability could affect growth after passaging.

RESULTS

Record your measurements and observations below. Show the $C_1V_1 = C_2V_2$ calculation and the fresh-medium calculation with units. Add the follow-up count when it is available.

Cell identity, passage, source and new vessel IDs: _____

Appearance and medium condition before passage: _____

Clear and blue counts in four squares: _____

Dye and any additional sample dilution: _____

C_1 measured living cells/mL and percent viability: _____

C_2 target living cells/mL and V_2 final volume: _____

V_1 calculated transfer and fresh-medium volumes: _____

Actual transfer and medium volumes; medium lot: _____

Calculated starting density and follow-up count: _____

Date, operator identities, and deviations: _____

Record your measurements and observations below. Show the $C_1V_1 = C_2V_2$ calculation and the fresh-medium calculation with units. Add the follow-up count when it is available.

Date: _____ Student: _____ Partner: _____

Cell identity: _____ Source passage: _____ New passage: _____

Source vessel ID: _____ Receiving vessel type and ID: _____

Assigned SOP or product sheet: _____ Allowed density (cells/mL): _____

Appearance clumping and medium condition before passage: _____

Raw cell counts

Sample or count time	Cell type	Square 1	Square 2	Square 3	Square 4
Before passage	living clear				
Before passage	dead blue				
Follow up	living clear				
Follow up	dead blue				

Dye dilution factor: _____ Additional sample dilution factor: _____

Total counting dilution factor: _____ Counting problems or repeat reason: _____

Density and transfer plan

Measurement	Value and units
C1 measured living cells/mL	
Viability before passage (%)	

C2 target living cells/mL	
V2 planned final culture volume (mL)	
V1 calculated source culture transfer (mL)	
Calculated fresh medium volume (mL)	
Actual source culture volume transferred (mL)	
Actual fresh medium added (mL)	
Actual final culture volume (mL)	
Calculated starting density (living cells/mL)	

Calculations

Show $V1 = C2 \times V2 \div C1$ and fresh medium = $V2 - V1$. Include units. Use actual volumes to calculate the actual starting density.

Calculation checked by: _____ Date and time: _____

Complete medium name and lot: _____

Transfer date and time: _____ Incubator ID and location: _____

Incubation conditions: _____

Student work record

Student name or ID	Steps performed	Date and time

If complete medium replacement was required: procedure reference _____

Actual centrifugation force (× g): _____ Time (min): _____ Cell loss if observed: _____

Use N/A for steps not performed.

Follow up culture record

Observation date and time	Living cells/mL	Viability (%)	Appearance clumping and medium condition	Action taken

Deviations unexpected observations and actions taken

CONCLUSIONS

1. Did the measured culture density justify passaging under the assigned THP-1 instructions? Explain with your actual result and the specified limit.
2. Compare your calculated starting density with the measured density at the next observation. Explain a reason they could differ, including the time that passed between measurements.
3. How would failing to mix the source culture immediately before sampling and transferring affect the count and the new vessel's starting density?
4. Under what circumstances would a complete medium replacement be needed instead of a routine dilution, and what additional measurements or records would that require?

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10: Plating THP-1 and NHDF Cells for ELISA and Scratch Assays

Learning Objectives

- Compare plating needs for cells that grow in suspension and cells that attach to the plate.
- Calculate living cells and suspension volumes for each assigned well.
- Explain why starting cell numbers, incubation time, and control wells should be consistent.
- Identify when plated cells are ready for medium collection or a gap assay.

BACKGROUND

Plating is the process of placing a measured number of living cells into culture wells. Consistent starting cell numbers and medium volumes help make comparisons between treatments more reliable. This exercise prepares THP-1 cells for measurement of proteins released into the medium and NHDF cells for a scratch assay. Each cell type needs different plating conditions because THP-1 cells normally grow in suspension, while NHDFs attach to a surface.

THP-1 cells are a human cell line that normally grows in **suspension**, meaning the cells are dispersed in culture medium rather than attached to the plate. Their medium may later be tested by **ELISA** (enzyme-linked immunosorbent assay), a method that uses antibody binding to measure a specific protein. Cells release molecules into the surrounding liquid. After incubation, this **conditioned medium**, or medium that has been in contact with cells, is collected for testing. The culture plate is not necessarily the same plate used to perform the ELISA. The culture plate and working volume must support healthy cells and provide enough sample for the assay.

A **control well** provides a comparison for the treated cells. Depending on the experiment, it may contain untreated cells, cells exposed to the treatment solvent, or medium alone. **Replicate wells** are separate wells prepared under the same condition; they help show how consistent the results are. Starting with similar numbers of living cells and using the same collection time are important because unequal cell numbers can change the amount of protein collected even when each cell releases the same amount. **Cell viability**, the proportion of cells that are alive, also helps explain differences between samples.

Normal human dermal fibroblasts (NHDFs) are cells from human skin connective tissue. They attach to **tissue-culture-treated plastic**, a surface prepared to promote cell attachment. A **scratch assay** follows the closure of a gap made in a cell layer. It begins with a nearly continuous **monolayer**, or one layer of attached cells. **Confluence** is the fraction of the growth surface covered by cells. The cells must reach the required confluence before scratching so the starting gap is clear and changes in its size can be compared.

Images taken at specified times show how the open area changes. Gap closure can reflect both **cell migration**, or movement of cells into the gap, and **proliferation**, an increase in cell number through division. Cell viability and growth must therefore be considered when interpreting the results. Uneven plating or open spaces elsewhere in the monolayer can make gap closure harder to measure.

The correct starting number of NHDFs depends on the cell lot, **passage number** (the number of times the culture has been transferred to a new vessel), plate surface area, and time available before scratching. A **pilot plating test**, a small trial of the planned starting density, helps determine whether the cells will form a suitable monolayer. Gentle mixing during dispensing and even distribution across the well help produce consistent cultures. Careful records of cell counts, volumes, and plating times allow later ELISA and scratch-assay results to be linked to the starting conditions.

MATERIALS

- Healthy THP-1 and NHDF cultures and their respective complete media
- Tissue-culture-treated 96-well round-bottom plate for THP-1 cultures, if approved for the assay
- Tissue-culture-treated 24-well plate for NHDF cultures
- Sterile tubes, pipettes, and filtered tips
- Cell counter or hemocytometer and viability dye

- Appropriate reagents for passaging adherent NHDFs
- Plate map and labeling supplies
- Incubator and microscope

METHODS

Prepare and count cells

1. Draw a **plate map** (a diagram showing what goes in each well) with all treatments, matched controls, and **replicate wells** (multiple wells prepared under the same condition). Record the intended collection or scratch time. Keep each treatment's starting volume and number of living cells consistent.
2. Prepare each culture using the laboratory's approved procedure. Collect THP-1 cells from suspension and detach NHDFs by their approved method. If either cell type is collected as a **cell pellet** (cells concentrated at the bottom of a tube), gently resuspend it in its own complete medium.
3. Measure each suspension's **viable cell concentration** (living cells per milliliter). Calculate suspension volume per well = desired living cells per well ÷ living cells per milliliter. Prepare enough suspension for all assigned wells and a small allowance for liquid left in the pipette. Record the calculation with units.

Plate THP-1 cells for a secreted-protein ELISA

4. Choose the THP-1 starting density and culture time from the experiment plan. ATCC recommends 2×10^5 to 4×10^5 viable cells/mL for THP-1 (TIB-202) in general culture; the class assay may require a different validated density.
5. For a 150 μ L culture volume, calculate living cells per well = living cells/mL $\times$ 0.150 mL. For example, 2×10^5 viable cells/mL gives 30,000 viable cells per well. Calculate how much cell suspension and medium are needed to reach 150 μ L in each well.
6. Mix the THP-1 suspension gently and repeatedly as you dispense so the cells do not settle. Add the same final volume to each experimental well. Include medium-only wells when required to assess background signal.
7. Incubate for the planned treatment period. Before collecting conditioned medium for ELISA, record the cell number or viability when feasible. Remove cells from collected medium and store samples according to the approved assay plan and kit instructions.

Plate NHDFs for the scratch assay

8. Use an instructor-approved pilot density expected to produce a nearly confluent, healthy NHDF monolayer at the planned scratch time. Calculate the needed cell suspension volume from the measured living-cell concentration.
9. Dispense NHDFs into assigned wells of a 24-well plate. Bring each well to approximately 1 mL with complete NHDF medium if this matches the plate and class procedure. Move the plate gently front-to-back and side-to-side to distribute cells evenly.
10. Incubate without disturbing the plate. At the planned time, often the next day, check attachment, cell shape, viability, and confluence. Proceed to scratching only when the monolayer meets the experiment's confluence criterion. Record wells with gaps or uneven growth.

EXERCISE Plating THP-1 and NHDF Cells for ELISA and Scratch Assays **NAME**_____

EXPECTATIONS

1. If one THP-1 well receives half the intended number of living cells, how might its measured protein concentration compare with a correctly plated well? Explain what else could affect that comparison.
2. What would you expect a scratch assay to show if an NHDF well has only 50% confluence before the gap is made? How would this affect interpretation of gap closure?

RESULTS

Complete the plate map and record the culture identity and passage, measured living cells/mL, target cells per well, calculations, cell suspension and total volume per well, medium lot, plating time, and treatment. For THP-1, record cell density or viability at

collection and the conditioned-medium sample ID. For NHDF, record attachment, morphology, and confluence before scratching, with representative images when available. Record any departures from the plan.

Student: _____ Partner: _____ Date: _____

Use short condition codes in the maps. Identify treatments, controls, and replicates in the key. Mark unused wells with an X.

THP-1 plate map (96 wells)

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Plate ID: _____ Plating date and time: _____

NHDF plate map (24 wells)

	1	2	3	4	5	6
A						
B						
C						

D						
---	--	--	--	--	--	--

Plate ID: _____ Plating date and time: _____

Plate map key

Code	Cell type / plate ID	Treatment or control (include dose and units)	Well IDs / replicates

Cell preparation and plating calculations

Culture and material record

Record	THP-1	NHDF
Culture ID / cell lot		
Passage number		
Complete medium / lot		
Measured living cells/mL		
Viability (%)		
Target living cells per well		
Target final volume per well (mL)		
Number of assigned wells		
Planned collection or scratch date / time		

Show calculations with units. Cell suspension volume per well = target living cells per well ÷ measured living cells/mL. Medium volume per well = final volume – cell suspension volume. For THP-1, living cells per well = target living cells/mL × final volume in mL.

THP-1 calculations, including total suspension needed and dispensing allowance:

NHDF calculations, including total suspension needed and dispensing allowance:

Plating volumes by condition

Cell type / code / wells	Calculated suspension per well (μL)	Actual suspension per well (μL)	Actual medium per well (μL)	Actual total per well (μL)

Record separate rows when wells receive different volumes.

Follow-up observations

THP-1 conditioned-medium collection

Well / code / replicate	Collection date / time	Cell density (living cells/mL) or cell number with units	Viability (%)	Medium sample ID

Record only measurements made. Use N/A when a count or viability measurement was not performed.

Medium processing method: _____

Sample storage conditions and location: _____

THP-1 appearance or collection notes: _____

NHDF confluence criterion required before scratching (%): _____

NHDF check before scratching

Well / code / replicate	Observation date / time	Attachment / cell shape / viability observations	Confluence (%)	Uneven areas / ready to scratch?	Image ID if available

Representative image file names or attachment IDs: _____

Departures from the plan and actions taken

CONCLUSIONS

1. Were THP-1 wells seeded at comparable viable cell numbers? How might a difference affect ELISA results?
2. Did NHDF wells form a suitable monolayer for a scratch assay? Support your answer with observations.
3. How would low cell viability change interpretation of a secreted-protein measurement or apparent wound closure?
4. What change to the next plating plan would improve consistency, and why?

11: Reverse Transcription and qPCR

Learning Objectives

- Distinguish cell lysates from conditioned medium.
- Explain why samples are kept cold during protein extraction.
- Prepare clarified protein lysates and measure their protein concentration.
- Describe why an extracellular vesicle preparation requires additional controls before a protein can be identified as vesicle associated.

BACKGROUND

After treatment with hTERT siRNA, NAMPT mRNA, or resveratrol, **mesenchymal stromal cells (MSCs)** may change the amounts of certain proteins they make. MSCs are cultured cells that can form several types of connective tissue cells. Measuring protein helps test whether a change in RNA is followed by a change in protein. RNA and protein measurements answer related questions, but one does not replace the other.

Cell lysis breaks open cells to release their contents. The resulting **cell lysate** is a mixture of proteins and other cell materials. **Conditioned medium** is culture medium collected after cells have grown in it. It may contain proteins released by cells, **extracellular vesicles (EVs)**, and material from damaged cells. EVs are small particles surrounded by a membrane that cells release. Cell lysate and conditioned medium are different sample types. Collect, label, and report them separately.

RIPA buffer is a solution that extracts many cellular proteins. It contains **detergents**, molecules that help disrupt membranes and bring proteins into solution. The buffer does not recover every protein equally well. Its ingredients can also affect later tests, so choose a protein assay that works with the exact buffer used. Keep the buffer amount and handling similar for comparable cultures.

Proteases are enzymes that break down proteins. When cells are opened, these enzymes can damage the proteins being studied. **Protease inhibitors** reduce their activity, while keeping samples cold slows many reactions. **Phosphatases** remove phosphate groups from proteins. If the experiment measures **phosphorylation**, the attachment of phosphate groups to proteins, add phosphatase inhibitors as directed. Protecting the sample during collection helps preserve the state you intend to measure.

After lysis, centrifugation separates material that does not dissolve from the liquid. The **pellet** is the material collected at the bottom of the tube. The **supernatant** is the liquid above it. Moving this liquid to a clean tube produces a **clarified lysate**, an extract with much of the solid material removed. Avoid disturbing the pellet when transferring the liquid.

A **BCA protein assay** is a color-based test that estimates total protein concentration. A **blank** contains the assay ingredients and sample buffer but no added sample protein. It helps account for background from the buffer. Known protein standards are used to relate the measured signal to protein concentration. The concentration lets you prepare equal amounts of total protein for a later **Western blot**, a test that separates proteins and detects a selected protein with antibodies. Equal loading improves the comparison but does not replace other quality checks.

Finding a protein in conditioned medium does not show how it arrived there. Cells may release it by secretion, injury, or shedding vesicles. **Cell viability**, the proportion of cells that remain alive, helps explain material released by damaged cells. The optional EV workflow removes intact cells and some debris before a separate isolation step. The final preparation may still contain **non-vesicular material**, proteins or particles that are not part of EVs.

Exosomes are EVs formed through a specific pathway inside cells. An isolation method alone does not prove that the collected EVs are exosomes. Detecting NAMPT in an EV preparation also does not prove that it is inside the vesicles or attached to their surface. Additional separation and location tests are needed. Record collection time, sample type, starting cell number or culture area, lysis volume, and protein concentration. These details help you judge whether a difference reflects protein changes, cell loss, or sample handling.

MATERIALS

- Treated and control MSC cultures
- Ice-cold phosphate-buffered saline (PBS)
- RIPA buffer
- Protease inhibitors
- Phosphatase inhibitors, if needed for the planned analysis
- Cell scraper
- Labeled microcentrifuge tubes
- Pipettes and tips
- Ice bucket with ice
- Refrigerated microcentrifuge
- Rocker or rotating mixer
- BCA protein assay kit or another assay compatible with the lysis buffer
- Plate reader or spectrophotometer
- For the optional conditioned-medium extension:
 - Conditioned medium from treated and control cultures
 - Conical centrifuge tubes
 - Refrigerated centrifuge
- An EV isolation method approved for the class

METHODS

Collect and lyse adherent MSCs

1. Label a tube for each treatment and control sample. Keep the tubes and prepared RIPA buffer cold.
2. Add protease inhibitors, and phosphatase inhibitors if needed, to the RIPA buffer according to the inhibitor instructions.
3. Remove the culture medium from each MSC culture. If conditioned medium will be studied, collect it in a separate labeled tube before washing the cells.
4. Gently rinse each cell layer with cold PBS to remove remaining medium and serum proteins. Remove the PBS.
5. Add enough prepared RIPA buffer to cover the cell layer, using the same buffer volume for comparable cultures.
6. Scrape the cells and transfer each suspension to its labeled tube.
7. Incubate the samples on ice for about 30 minutes, mixing occasionally.
8. Centrifuge the lysates at about $16,000 \times g$ for 10–20 minutes at 4 °C, or use the conditions specified by the laboratory's validated protocol.
9. Carefully transfer each supernatant to a new labeled tube without disturbing the pellet.
10. Measure protein concentration using a BCA assay or another assay confirmed to be compatible with the RIPA formulation. Use the same buffer to prepare the assay blank.
11. Keep the clarified lysates cold during use. Store them according to the laboratory's protocol if analysis will occur later.

Optional extension: collect conditioned medium for EV analysis

1. Transfer conditioned medium to labeled tubes without disturbing the cell layer.
2. Centrifuge at $300 \times g$ for 10 minutes to remove intact cells. Transfer the supernatant to new tubes.
3. Centrifuge at $2,000 \times g$ for 30 minutes at 4 °C to reduce remaining cell debris. Transfer the supernatant to new tubes without disturbing the pellet.

4. Isolate EVs using the laboratory's validated method and the equipment or kit instructions. Recovery and purity can differ between methods.
5. If the later assay calls for an EV lysate, add an appropriate volume of cold lysis buffer to the isolated preparation and process it according to the validated protocol.
6. Record the volume of conditioned medium collected, the number of cells or culture area, and any differences in cell viability. These details are needed to compare EV preparations fairly.

EXERCISE Protein Collection and Cell Lysis NAME_____

EXPECTATIONS

1. If NAMPT mRNA transfection increases cellular NAMPT protein, how should the NAMPT signal in treated cell lysates compare with the control?
2. If an EV preparation contains NAMPT, what additional evidence would you need before concluding that NAMPT was carried by EVs?

RESULTS

Record the treatment, sample type, collection time, lysis volume, and protein concentration for each sample. For conditioned medium, also record the starting medium volume and the EV isolation method. Note any treatment that visibly reduced cell number or viability.

Student name: _____ Partner: _____

Date: _____ Group: _____ Experiment ID: _____

Record units with every measurement. Use N/A for tests not performed. Add rows or attach raw data as needed.

Cell culture and collection record

Sample ID and treatment	Cell number or culture area	Viability (%)	Collection time	Lysis volume (μL)

Protein assay standards

Standard ID	Protein concentration and units	Signal 1	Signal 2	Mean signal

Protein concentration and yield

Sample ID	Dilution factor	Mean signal	Protein conc. (μg/μL)	Total protein (μg)

Optional conditioned medium and EV record

Sample ID	Medium volume (mL)	EV method	Recovered volume (μL)	Observations

Calculations

Total protein = concentration × lysate volume. Record the assay dilution factor.

Observations, excluded data, and changes from the procedure

CONCLUSIONS

1. Why is it important to compare equal amounts of lysate protein when examining cellular NAMPT or hTERT?
2. How could incomplete removal of culture medium affect a cell lysate measurement?
3. How might reduced cell viability affect interpretation of protein found in conditioned medium?
4. What can an EV preparation tell you about NAMPT, and what can it not establish on its own?

12: RNA Extraction from MSCs Using RNA-BEE

Learning Objectives

- Explain how SDS-PAGE separates proteins.
- Describe the purpose of protein transfer, blocking, and antibody incubation steps.
- Use a molecular weight ladder and controls to interpret an immunoblot.
- Compare protein signals after adjusting for differences in sample loading.

BACKGROUND

SDS-PAGE stands for sodium dodecyl sulfate–polyacrylamide gel electrophoresis. It separates proteins mainly by size. **Immunoblotting**, also called Western blotting, then uses antibodies to detect a selected protein. In this exercise, the target may be NAMPT or hTERT. These methods help compare protein signals in treated and control samples. A reliable comparison requires measured protein amounts, suitable controls, and consistent sample handling.

A **cell lysate** contains materials released when cells are broken open. It is mixed with **sample loading buffer**, a solution that prepares proteins for the gel. The buffer contains **SDS**, a detergent that helps unfold proteins and gives them a negative charge. A **reducing agent** may be added to break certain sulfur-to-sulfur bonds in proteins. Use it only when required by the target and antibody protocol. These treatments reduce differences in protein shape so that size has a larger effect on movement through the gel. Some proteins still behave differently.

A **polyacrylamide gel** is a porous material that slows proteins as they move through it. Each sample is loaded into a separate **lane**, a track through the gel. During **electrophoresis**, charged molecules move in an electric field. SDS-coated proteins move toward the positive electrode. Smaller proteins usually move farther than larger ones. A **molecular weight ladder** contains proteins of known sizes and gives a size reference. A band's position alone cannot prove which protein it contains.

Load the same measured mass of total protein in each sample lane. The volume needed depends on the lysate concentration. For example, 4 μL of a lysate containing 5 $\mu\text{g}/\mu\text{L}$ supplies 20 μg of protein. Equal volumes do not always contain equal protein amounts. Record the sample order so each band can be linked to the correct treatment.

After separation, **transfer** moves proteins from the gel onto a **membrane**, a thin sheet that binds proteins. An electric current drives this movement. Correct orientation is important because proteins must move toward the membrane. Air bubbles can block contact and leave areas with poor transfer. A **total-protein stain** reveals many proteins in each lane. It helps check transfer and compare loading before antibody detection.

A **blocking solution** fills unused binding sites on the membrane to reduce unwanted antibody binding. A **primary antibody** binds the protein of interest. A **secondary antibody** binds the primary antibody and carries a label that helps detect it. The secondary antibody must match the primary antibody. Follow the antibody instructions for blocking, dilution, incubation, and washing. Washing removes unbound antibodies and helps reduce background.

In this exercise, the secondary antibody carries **horseradish peroxidase (HRP)**, an enzyme used for detection. A **chemiluminescent substrate** reacts with HRP to produce light. An imaging system records the light as a band. The signal depends on the target protein, antibody binding, and imaging settings. An **overexposed band** reaches the image's signal limit, so its brightness cannot be used for a reliable comparison. Choose an exposure that keeps the signal within the system's measurable range.

Positive controls are samples expected to contain the target signal. **Negative controls** are samples expected to lack it. These controls and the expected band size help judge whether the band is the intended protein. **Nonspecific binding** occurs when an antibody binds something other than its target. Extra bands or strong background may therefore need further checks before interpretation.

Band intensity is the measured signal from a band. Use a suitable **loading reference**, such as total-protein staining or a protein shown to stay stable under the treatments, to adjust the target signal. This adjustment is called **normalization**. Compare the

normalized values with the matched control. A difference supports a change in detected protein under these conditions, but the blot alone does not prove how the treatment caused that change.

MATERIALS

- Clarified protein lysates and their measured concentrations
- Protein standards or controls specified by the instructor
- 4× sample loading buffer
- Reducing agent, if required
- SDS-PAGE gel
- Running buffer
- Protein molecular weight ladder
- Gel electrophoresis tank and power supply
- Heating block
- PVDF membrane and methanol, if required for that membrane
- Transfer buffer, filter paper, and sponges
- Transfer apparatus
- Ponceau S or another total-protein stain
- **TBST** wash buffer, Tris-buffered saline with Tween detergent
- Blocking solution appropriate for the antibodies
- Primary antibody against the target protein
- Compatible HRP-conjugated secondary antibody
- Chemiluminescent detection reagent
- Imaging system and image analysis software

METHODS

Prepare and separate the samples

1. Use the measured lysate concentrations to calculate the volume needed to load the same mass of protein in each lane. For example, to load 20 µg from a lysate containing 5 µg/µL, use 4 µL of lysate.
2. Add sample loading buffer to its specified final concentration. Add reducing agent if required. Bring samples to the same final volume using a suitable buffer.
3. Heat samples only at the temperature and for the time recommended for the target protein, antibody, and sample buffer. Some proteins may clump together or lose antibody-binding sites if overheated.
4. Briefly centrifuge the tubes to collect liquid at the bottom.
5. Place the gel in the electrophoresis tank and add running buffer according to the gel instructions.
6. Load the molecular weight ladder, controls, and equal protein masses of the experimental samples.
7. Run the gel using the voltage and stopping point recommended for the gel and tank. Record the conditions used.

Transfer and detect the proteins

1. Prepare the membrane according to its manufacturer's instructions. If the PVDF membrane requires methanol wetting, complete that step before soaking it in transfer buffer.
2. Soak the gel and transfer materials in transfer buffer.
3. Assemble the transfer stack in the order required by the transfer apparatus. Position the membrane on the positive-electrode side of the gel so proteins move from the gel onto the membrane. Remove air bubbles between the gel and membrane.
4. Transfer proteins using the validated settings for the gel, membrane, buffer, and transfer apparatus. Record the settings; a single current and duration will not suit every setup.

5. Stain the membrane with Ponceau S or another total-protein stain to check transfer and compare lane loading. Record an image if the stain will be used for normalization.
6. Rinse the stain from the membrane. Block it using a solution that works with the primary antibody. Follow the antibody instructions when choosing milk or **BSA**, bovine serum albumin.
7. Incubate the membrane with the primary antibody at its recommended dilution and temperature.
8. Wash the membrane with TBST according to the laboratory protocol.
9. Incubate with the compatible HRP-conjugated secondary antibody at its recommended dilution.
10. Wash the membrane again to reduce unbound secondary antibody.
11. Add chemiluminescent detection reagent and capture images. Use exposure settings that keep the bands within the measurable range of the imaging system.
12. Measure band intensity and normalize each target band to the selected loading reference. Compare normalized values with the appropriate control group.

EXERCISE SDS-PAGE and Immunoblotting NAME_____

EXPECTATIONS

1. If hTERT siRNA reduces hTERT protein, what change would you expect in the hTERT band after adjusting for lane loading?
2. How could unequal protein loading make an unchanged protein appear to increase or decrease?

RESULTS

Include a labeled blot image identifying the molecular weight ladder, controls, and treatment lanes. Record the approximate position of the target band, the loading-reference signal, and the normalized band intensity for each sample. Report results relative to the appropriate control.

Student name: _____ Partner: _____

Date: _____ Group: _____ Experiment ID: _____

Record units with every measurement. Use N/A for tests not performed. Add rows or attach raw data as needed.

Target: _____ Primary antibody/lot: _____ Dilution: _____

Secondary antibody/lot: _____ Dilution: _____ Exposure: _____

Lane map and sample preparation

Lane	Sample ID and treatment	Protein conc. ($\mu\text{g}/\mu\text{L}$)	Protein mass (μg)	Lysate volume (μL)

Sample loading mixture

Sample ID	Lysate (μL)	Loading buffer (μL)	Reducing agent (μL)	Other buffer (μL)	Total (μL)

Band measurements

Sample ID	Target size (kDa)	Target signal	Loading reference signal	Normalized signal	Relative to control

Run and detection settings

Stage	Reagent or equipment ID	Settings and time

Calculations

Lysate volume = desired protein mass ÷ concentration. Show the loading-reference adjustment.

Observations, excluded data, and changes from the procedure

Attach the labeled blot. File name or attachment ID: _____

CONCLUSIONS

1. Do the band position and control lanes support identification of the target protein? Explain.
2. Did treatment change the normalized hTERT or NAMPT signal? Use the measured results to support your answer.
3. Did the total-protein stain or housekeeping protein reveal unequal loading or transfer? How did you address it?
4. What is one limitation of this immunoblot, and what follow-up measurement would strengthen your conclusion?

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13: NAMPT mRNA Transfection

Learning Objectives

- Explain how lipid reagents help deliver mRNA into cells.
- Distinguish transient mRNA expression from plasmid DNA expression.
- Identify controls and measurements needed to evaluate a transfection.

BACKGROUND

Transfection (introducing nucleic acids into cells) allows us to change what cells make and then observe the result. **Messenger RNA (mRNA)** (an RNA copy of genetic instructions that a cell can translate into protein) gives the cell a template for making a specific protein. In this exercise, the introduced mRNA carries instructions for making NAMPT. This is different from **small interfering RNA (siRNA)** (a short RNA molecule used to reduce the amount of a selected mRNA). Supplying NAMPT mRNA is intended to increase the opportunity to make NAMPT protein; an siRNA experiment is generally designed to reduce expression of its target.

Mesenchymal stromal cells (MSCs) (cells grown in culture that can form several connective tissue cell types) receive mRNA encoding **NAMPT** (nicotinamide phosphoribosyltransferase, an enzyme that helps recycle nicotinamide to make NAD⁺). **NAD⁺** (nicotinamide adenine dinucleotide, a molecule used in many cellular energy and repair reactions) is important to cell function. An increase in NAMPT protein might affect this pathway, but the outcome must be measured: adding mRNA alone does not establish that the protein or NAD⁺ level increased.

RNA is easily broken down outside cells, and its size and electrical charge make it difficult to pass directly through the cell membrane. A **lipid transfection reagent** (a preparation of fat-like molecules that helps deliver nucleic acids into cells) is mixed with mRNA to form **mRNA-lipid complexes** (particles containing both the mRNA and the delivery reagent). Following the reagent instructions for mixing order, amount, and waiting time helps the complexes form reproducibly. After the complexes enter cells, mRNA that reaches the **cytoplasm** (the region of the cell outside the nucleus) can be read by **ribosomes** (cell structures that build proteins from mRNA instructions). Delivery can vary between wells and can stress cells, so record cell appearance and **viability** (the proportion of cells that remain alive).

Expression from introduced mRNA is generally **transient** (lasting for a limited time), because the mRNA is gradually degraded and does not ordinarily become part of the cell's DNA. **Plasmid DNA** (a circular DNA molecule that can carry a gene) follows a different route: it must reach the **nucleus** (the compartment containing the cell's chromosomes) before the cell can transcribe it into mRNA. Plasmid expression may also be temporary; plasmid delivery alone does not show that DNA integrated into the genome. The treatment and measurement times matter because RNA, protein, and downstream effects may reach their highest levels at different times.

Controls help separate the effect of the NAMPT sequence from the effect of the delivery process. A **control mRNA** (an mRNA chosen for comparison that does not encode NAMPT) helps show how cells respond to receiving mRNA and lipid. **Reagent-only** wells (cells exposed to lipid reagent without mRNA) can reveal effects of the delivery reagent, while untreated wells show the culture's baseline condition. **Replicates** (independently prepared wells receiving the same treatment) help show whether a result is consistent. Use the assigned assay to measure NAMPT RNA, and measure NAMPT protein separately if the class has a validated protein assay. A high RNA signal does not prove protein production, and a change in NAD⁺ alone does not identify its cause. Compare each result with its matched control and consider changes in cell number or viability.

MATERIALS

- MSCs
- Six-well culture plates
- NAMPT mRNA
- Lipid reagent suitable for mRNA transfection and MSCs
- Medium specified by the transfection reagent instructions
- Antibiotic-free culture medium
- Complete culture medium

- Appropriate control mRNA and untreated or reagent-only controls
- Cell culture equipment
- Materials for cell viability and NAMPT expression measurements

METHODS

1. Using the class cell plating procedure, seed MSCs in labeled six-well plates with the assigned volume of medium. Plan for the cells to reach the confluence range specified for the selected mRNA reagent on transfection day. Record cell passage, plating density, and well IDs.
2. Make a well map that assigns NAMPT mRNA, an appropriate control mRNA, and untreated or reagent-only controls as directed. Include replicates. Write down the planned sampling times and assays before preparing mixtures.
3. Read the selected transfection reagent instructions for the mRNA amount, lipid amount, dilution medium, and final culture volume for a six-well plate. Calculate the amounts needed for all assigned wells plus the approved pipetting allowance. Dilute mRNA and lipid separately, and prepare matched control mixtures the same way.
4. Combine the diluted mRNA with the diluted lipid reagent in the order specified for that product. Allow the mRNA-lipid complexes to form for the stated time. Record the preparation and addition times.
5. Inspect the MSCs for healthy appearance and the required confluence. Change the medium only if directed by the reagent procedure. Add the assigned mixture to each labeled well, distribute it gently, and record the actual amounts. Keep all wells at the assigned final culture volume.
6. Return the plate to its assigned incubator conditions. Observe the cells at the planned time and change medium if the reagent and cell-specific procedure requires it. Record cell appearance and any signs of stress.
7. At the planned collection time, measure cell viability and collect samples using the separate assay procedures. RT-qPCR measures NAMPT RNA, while an immunoassay or immunoblot can measure NAMPT protein if validated for this experiment. If NAD⁺ is measured, record it as a separate outcome. Compare each measurement with the appropriate control and account for cell number or viability when required by the assay.

EXERCISE NAMPT mRNA Transfection NAME _____

EXPECTATIONS

1. Predict how NAMPT RNA and NAMPT protein measurements might compare between NAMPT mRNA wells and control mRNA wells if delivery and translation succeed.
2. Predict how a large decrease in cell viability could complicate your interpretation even if NAMPT RNA appears higher in treated wells.

RESULTS

Complete the record below and attach the well map and assay data. Report NAMPT RNA, NAMPT protein, and NAD⁺ as separate measurements when available. Show how each treated result was compared with its matched control.

Culture and transfection plan

Student: _____ Partner: _____ Date: _____

MSC identity/source: _____ Passage: _____ Plating density and units: _____

Plate ID: _____ Plating date/time: _____ Confluence (%): _____

mRNA ID/lot: _____ Lipid reagent/lot: _____ Medium/lot: _____

Reagent instruction reference: _____ Incubator conditions: _____

Planned sampling times and assays: _____

Record units and actual observations. Use N/A for measurements not performed.

Six well treatment map

Well	Treatment / control	Replicate	Sample ID
A1			
A2			
A3			
B1			
B2			
B3			

Mixture preparation

Treatment	mRNA amount and units	Lipid (μL)	Dilution medium (μL)	Final well volume (mL)

Transfection and assay results

Student: _____ Partner: _____ Date: _____

Complex preparation time: _____ Complex formation interval: _____

Mixture addition time: _____ Medium change time or N/A: _____

Assay method/units: RNA _____ Protein _____ NAD $\diamond$ _____

Normalization method and matched control: _____

Record units and actual observations. Use N/A for measurements not performed.

Culture observations

Well / sample ID	Date / time after addition	Medium / appearance	Viability (%)	Collection time

--	--	--	--

Separate assay results

Sample / replicate	Matched control ID	NAMPT RNA / units	NAMPT protein / units	NAD $\diamond$ / units

Calculations and observations

Show reagent amounts for all assigned wells and the approved pipetting allowance. Show how each measured result was normalized, if required, and compared with its matched control. Attach raw assay data and calculations.

Observations deviations and corrective actions

Raw assay data / attachment IDs: _____

CONCLUSIONS

1. What evidence supports delivery of NAMPT mRNA? What additional evidence would show that the cells produced more NAMPT protein?
2. Did transfection affect cell viability? How does that affect your interpretation of the NAMPT results?
3. If NAD $\diamond$ was measured, did it change along with NAMPT protein? Explain what these measurements do and do not establish. If NAD $\diamond$ was not measured, explain what conclusion remains open.
4. Which control was most useful for interpreting your results, and why?

14: Tangential Flow Filtration of MSC Conditioned Medium

Learning Objectives

- Trace the feed, retentate, and permeate paths through a hollow-fiber TFF system.
- Explain how concentration differs from diafiltration.
- Calculate a concentration factor and a diafiltration volume.
- Identify measurements needed to evaluate EV enrichment and recovery.

BACKGROUND

Mesenchymal stromal cell (MSC) conditioned medium is growth medium collected after MSCs have released material into it. It may contain **extracellular vesicles (EVs)**, small particles surrounded by a cell-derived membrane, as well as dissolved proteins and cell debris. It can also contain **lipoproteins**, particles made of fats and proteins. **Exosomes** are EVs formed through a specific pathway inside cells. Particle size or filtration alone cannot show that every collected EV is an exosome.

Tangential flow filtration (TFF) is a method in which liquid flows along a **membrane**, a thin barrier that lets some materials pass while holding back others. TFF can reduce the sample volume and exchange some of its liquid for another buffer. It does not produce a pure EV sample by itself. In **dead-end filtration**, liquid flows toward the membrane, where retained material can build up. In TFF, flow along the surface can reduce this buildup.

The **feed** is the starting liquid entering the filter module. The **permeate** is the liquid that passes through the membrane and leaves through a separate outlet. The **retentate** is the liquid and retained material that stay on the feed side and return to the reservoir. **Crossflow** is movement along the membrane surface. **Fouling** is buildup that blocks or changes membrane flow. Even with crossflow, fouling can affect processing and sample recovery.

A **hollow-fiber module** is a cartridge containing many small membrane tubes. Sample flows through the fibers while permeate crosses their walls. The proposed D02-E300-05-N module is listed as a 300 kDa mPES MidiKros unit with about 115 cm² of membrane area. Check the actual label and instructions before use. **Molecular weight cutoff (MWCO)** is a rating based on how the membrane retains test molecules. It is not a direct measurement of pore diameter. A 300 kDa rating does not guarantee that all EVs stay in the retentate or that all dissolved proteins pass through.

Concentration reduces sample volume by removing permeate. Retained material becomes more concentrated if it remains in the system. The **concentration factor** equals the starting feed volume divided by the final retentate volume. Reducing 250 mL to 25 mL gives a factor of 10. This volume change does not show how many EVs were recovered. **Recovery** compares the amount of a chosen material collected at the end with its starting amount. Some EVs may be lost to the filter, tubing, surfaces, or permeate. Measure the starting and final samples to evaluate recovery.

Diafiltration exchanges the liquid around retained material by adding fresh buffer while permeate leaves. At nearly constant retentate volume, one **diafiltration volume (DV)** equals the retentate volume. If the retentate is 25 mL, three DVs require about 75 mL of buffer. Small molecules that pass freely through the membrane become less concentrated during exchange, but complete removal is not guaranteed. Rinsing an empty cartridge after sample collection is a different step. Choose a buffer that works with the later analysis.

Feed flow, pressure, temperature, and processing time affect TFF performance. **Transmembrane pressure (TMP)** is the pressure difference that drives liquid across the membrane. Keep it within the limits for the actual setup. Pump speed alone does not show flow or pressure. Compare the starting medium, final retentate, and selected permeate samples using the tests available in class. A **medium-only process control** is medium processed without cells. It helps reveal background material from the medium or handling. Describe the sample as EV-enriched only when the test results support that description.

MATERIALS

- MSC conditioned medium and matched medium-only process control

- Refrigerated centrifuge and compatible tubes
- Optional 0.45 μm filter validated for the planned EV fraction
- Peristaltic pump and compatible tubing and pump head
- Hollow-fiber module D02-E300-05-N or the laboratory-approved alternative
- Manufacturer-specified sterile connectors, caps, tubing, and collection vessels
- Pressure measurement and control equipment required by the system
- PBS or another validated diafiltration buffer
- Clamps, labeling supplies, and graduated collection vessels
- Biosafety cabinet and approved disinfectant for aseptic assembly
- Temperature control, if required by the sample plan
- Materials for later EV testing of sample properties

METHODS

Prepare and clarify the medium

1. Label the starting medium, retentate, permeate, buffer, and control containers. Record the starting volume, cell culture conditions, and cell viability at collection.
2. Centrifuge conditioned medium at $300 \times g$ for 10 minutes to remove cells. Transfer the supernatant to a new container without disturbing the pellet.
3. Centrifuge the supernatant at $2,000 \times g$ for 30 minutes at 4 °C to reduce debris. Transfer the cleared supernatant to a new container.
4. If the experimental plan requires it, pass the sample through a validated 0.45 μm filter. Record the filter used; filtration can remove larger EVs and may reduce recovery. Do not assume it selects only exosomes.

Assemble, prime, and run the TFF system

1. Check the hollow-fiber module label, port diagram, tubing compatibility, allowed pressures and flows, and minimum working volume against the manufacturer instructions. Have the instructor verify the flow path before starting the pump.
2. Assemble the feed line from the starting vessel through the pump to the module feed port. Return the retentate outlet to the starting vessel. Route the permeate outlet to a separate labeled collection vessel. Follow the module's port diagram; do not identify ports only by their position on a drawing.
3. Clean external surfaces and assemble the flow path aseptically in the biosafety cabinet using sterile or appropriately prepared components. Do not rely on cabinet UV exposure to sterilize tubing or equipment. Keep electrical connections and the cabinet airflow configuration within the laboratory's approved setup.
4. Prime, flush, and condition the module with the volume and solution specified by the manufacturer and the laboratory protocol. Confirm correct pump direction, intact connections, and open flow paths. Direct the initial flush to waste, not to the sample vessel.
5. Introduce clarified conditioned medium and operate within the validated flow, pressure, and temperature limits for this module and sample. Record feed, retentate, and permeate pressures when the system supports them. Pump RPM alone is not a flow or pressure setting that works for every setup.
6. Collect permeate while recirculating retentate. Stop concentration at the planned retentate volume without allowing the reservoir to run dry. Record starting volume, retentate volume, permeate volume, processing time, and any visible fouling or leak.
7. If diafiltration is planned, add fresh buffer while collecting permeate at about the same rate so the retentate volume remains nearly constant. Process the specified number of DVs and record the actual buffer volume used. Do not treat a separate PBS flush after removing the sample as equivalent to diafiltration.
8. Recover the final retentate according to the laboratory protocol. If module recovery or a post-run flush is specified, collect and label those fractions separately. Clean or discard the flow path according to the module instructions.

EXERCISE Tangential Flow Filtration of MSC Conditioned Medium

NAME_____

EXPECTATIONS

1. If 250 mL of conditioned medium is reduced to 50 mL, what concentration factor do you expect? Does this factor alone show that EV recovery was high?
2. During constant-volume diafiltration, what would you expect to happen to freely permeating small molecules in the retentate?

RESULTS

Report the starting volume, final retentate volume, concentration factor, diafiltration buffer volume and DVs, sample temperature, operating measurements, and recovered fractions. Present EV and soluble-protein assay results for the starting medium and final retentate, and for permeate when measured.

Student name: _____ Partner: _____

Date: _____ Group: _____ Experiment ID: _____

Record units with every measurement. Use N/A for tests not performed. Add rows or attach raw data as needed.

Module ID/MWCO: _____ Membrane area: _____ Buffer/lot: _____

Pressure units for the operating log: _____ Allowed limits: _____

Fraction collection record

Fraction ID	Fraction type	Volume (mL)	Collection time	Storage conditions

TFF operating log

Elapsed time (min)	Flow and units	Feed pressure	Retentate pressure	Permeate pressure	Temperature (°C)

Concentration and diafiltration record

Starting volume (mL)	Final retentate (mL)	Concentration factor	Buffer added (mL)	DVs

Sample quality and recovery

Fraction ID	Particle conc. and units	Particle size and units	Protein conc. and units	EV/non-EV test result

Calculations

Concentration factor = starting volume ÷ final volume. DVs = buffer added ÷ constant retentate volume. Recovery (%) = final amount ÷ starting amount × 100.

Observations, excluded data, and changes from the procedure

CONCLUSIONS

1. What evidence shows that the sample was concentrated? What additional measurements are needed to calculate EV recovery?
2. Did diafiltration reduce soluble contaminants according to the available data? Explain.
3. How might prefiltration or membrane fouling affect the EV fractions recovered?
4. What testing of sample properties would be needed before describing the product as an EV-enriched preparation?

ATTRIBUTIONS:

International Society for Extracellular Vesicles. MISEV guidance: <https://www.isev.org/misev>

Repligen. Spectrum hollow-fiber filter configurator, D02-E300-05-N: <https://www.repligen.com/configurator/hollow-fiber>

Repligen. Tangential flow filtration guide: <https://www.repligen.com/unit-operat...ltration-guide>

15: ELISA for Cytokine Quantification

Learning Objectives

- Explain how a sandwich ELISA measures a cytokine in culture supernatant.
- Prepare a plate map with standards, blanks, controls, and sample replicates.
- Calculate cytokine concentration from a standard curve and account for sample dilution.
- Interpret cytokine changes while considering cell number and viability.

BACKGROUND

Cytokines are small proteins that cells release to communicate with other cells. They help coordinate **inflammation**, a response to injury or infection. In this exercise, THP-1 cells provide **culture supernatant**, liquid collected from above the cells. It may contain cytokines such as IL-1 β , IL-6, or IL-8. Measuring these proteins helps compare how cells respond to treatments. Each ELISA kit measures a specific target, so use a kit for the cytokine being studied.

A **sandwich enzyme-linked immunosorbent assay (ELISA)** traps a target protein between two antibodies and measures it using an enzyme signal. **Antibodies** are proteins that recognize specific features of a molecule. A **capture antibody** is attached to the plate and binds the target. A **detection antibody** binds a different part of that target. Washing removes material that has not bound. Incomplete washing can increase background signal.

Many kits use **horseradish peroxidase (HRP)**, an enzyme in the detection system, and **TMB substrate**, a chemical that changes color when HRP acts on it. A **stop solution** ends color development after the required time. A plate reader measures **absorbance**, the amount of light absorbed by the colored solution, often near 450 nm. Within the kit's working range, more captured cytokine usually produces more color. Absorbance alone is not a concentration.

A **standard curve** relates signals to known cytokine concentrations on the same plate. Sample signals are compared with this curve to estimate concentration, often in **picograms per milliliter (pg/mL)**, a unit for very small protein amounts. A **blank** measures background without added cytokine. Controls help reveal unexpected effects of the sample mixture. If the sample was diluted, multiply the concentration from the curve by the **dilution factor**, the ratio of final diluted volume to original sample volume. Values outside the tested curve range cannot be reported as exact concentrations.

Kits differ in reagent order, sample volume, timing, sensitivity, and working range. Follow the instructions for the exact kit used in class. Some kits combine steps that other kits perform separately. The **assay diluent** is the solution used to prepare standards or dilute samples. It matters because the sample liquid can affect antibody binding and signal. Do not use the concentration range or timing from a different kit.

Compare treated samples with the assigned controls, which may include untreated cells, cells receiving only a stimulus, and cells receiving only the treatment solvent. A lower cytokine concentration may reflect less release by cells. It may also reflect fewer living cells, a different medium volume, or interference from the treatment. Record cell number or **viability**, the proportion of living cells, and the culture volume. These measurements help explain the cytokine results.

MATERIALS

- Clarified THP-1 culture supernatants and matched control medium
- Cytokine-specific ELISA kit for IL-1 β , IL-6, or IL-8, including its instructions and standard
- Kit-specified assay diluent, wash buffer, detection reagents, substrate, and stop solution
- Compatible 96-well assay plate if not supplied by the kit
- Pipettes, tips, reservoirs, and plate seals
- Plate washer or appropriate aspiration setup
- Plate reader with the wavelength required by the kit
- Plate map, sample dilution record, and assay analysis software
- Appropriate chemical and biological waste containers

METHODS

Collect and prepare samples

1. At the specified treatment time, record the treatment, culture volume, viable cell number or another appropriate cell measure, and any visible differences in viability.
2. Collect the supernatant without disturbing the cells. Clarify it as directed by the ELISA kit or class protocol and transfer the cleared liquid into labeled tubes.
3. Assay fresh samples or store separate portions under conditions approved for the target molecule and kit, often frozen at -80°C for longer storage. Avoid repeated freeze-thaw cycles. Prepare any required sample dilutions in the kit-specified diluent.

Run the cytokine ELISA

1. Read the instructions for the exact kit and bring only the specified components to the required temperature. Do not assume that every reagent should reach room temperature.
2. Prepare a plate map showing standards, zero standard or blank, medium-only control, treatment controls, and each sample in duplicate or the replicate scheme specified by the class. Record sample dilutions.
3. Dissolve the kit standard and prepare the serial dilutions using its specified diluent and concentration range. Make a fresh standard curve for each assay plate.
4. Dispense standards, controls, and samples using the volumes and order stated by the kit. Keep pipetting time consistent across wells.
5. Complete the kit's capture, detection, enzyme, wash, and incubation steps in the exact order given. Some kits have fewer steps than a conventional sandwich ELISA. Aspirate to an approved waste container; do not invert the plate over a sink. Avoid letting wells dry between steps.
6. Add the specified substrate and allow color to develop for the kit-defined time. Add stop solution in the same well order and with consistent timing. Follow the safety instructions for the stop solution and waste.
7. Read the plate at the kit-specified wavelength and within the allowed time after stopping. Use a reference wavelength only when recommended and supported by the reader and kit.

Analyze the data

1. Check that the blank, standard curve, controls, and replicate variation meet the assay acceptance criteria. Plot standard concentration against absorbance using the fit recommended by the kit, often a four-parameter logistic curve.
2. Estimate sample concentrations from the standard curve only within the validated measurement range. Dilute and re-run samples above range when possible; report below-range samples appropriately rather than assigning an exact concentration.
3. Multiply the estimated from the standard curve concentration by the sample dilution factor to obtain the concentration in the original supernatant. If needed, calculate total cytokine in the collected volume as concentration $\times$ volume. Compare groups using a consistent basis and report the cell count or viability alongside cytokine results.

EXERCISE ELISA for Cytokine Quantification NAME_____

EXPECTATIONS

1. Predict how the IL-6 absorbance of an LPS-only sample will compare with an untreated control if LPS stimulates cytokine release. What result would you expect after the sample signals are converted to concentrations?
2. Predict what a treatment sample might show if the treatment reduces both THP-1 viability and cytokine release. What additional

measurement would help you interpret the result?

RESULTS

Show the plate map, standard curve, sample dilution factors, replicate absorbances, estimated from the standard curve concentrations, and final pg/mL values. Report results for all control groups and record any samples outside the curve range. Include the cell number or viability data used to interpret cytokine secretion.

Student name: _____ Partner: _____

Date: _____ Group: _____ Experiment ID: _____

Record units with every measurement. Use N/A for tests not performed. Add rows or attach raw data as needed.

Cytokine: _____ Kit/catalog ID: _____ Kit lot: _____

Read wavelength: _____ Read time: _____ Curve fit: _____

Standard curve data

Standard ID	Cytokine conc. (pg/mL)	Absorbance 1	Absorbance 2	Mean absorbance

Sample and control results

Sample ID	Dilution factor	Absorbance 1	Absorbance 2	Curve conc. (pg/mL)	Original conc. (pg/mL)

Culture measurements

Sample ID	Culture volume (mL)	Living cell count	Viability (%)	Total cytokine (pg)

ELISA plate map

	1	2	3	4	5	6	7	8	9	10	11	12
A												

B												
C												
D												
E												
F												
G												
H												

Plate map key / sample abbreviations: _____

CONCLUSIONS

1. Did the standards and controls support a valid measurement? Identify any results that should be repeated.
2. How did each treatment affect cytokine concentration compared with the correct control? Use numbers to support your answer.
3. Could differences in cell number, viability, or medium explain any observed change? Explain.
4. What additional control or experiment would strengthen a claim that an MSC EV preparation altered THP-1 inflammatory signaling?
5. Why must standards be prepared using the diluent specified by the kit?
6. Why might an absorbance value above the highest standard require dilution and a repeat measurement?
7. Why should a medium-only or EV-preparation control be included?
8. How does concentration in pg/mL differ from the total cytokine amount collected from a culture well?

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16: NHDF Wound Scratch Assay and Quality Checks

Learning Objectives

- Measure changes in a cell-free gap in an NHDF monolayer over time.
- Distinguish wound closure from cell migration alone.
- Explain the purpose and limits of mycoplasma, endotoxin, and microbial-growth checks.
- Interpret assay results alongside cell viability and quality-control data.

BACKGROUND

Normal human dermal fibroblasts (NHDFs) are cells from the connective tissue of human skin. They can move and divide during tissue repair. A **wound scratch assay** tracks how cultured cells fill a gap made in a cell layer. Students create a gap in a nearly continuous **monolayer**, a single layer of attached cells, and photograph the same locations over time. This simple model does not include the blood supply, immune cells, or all other features of a wound in living tissue.

Gap closure is the reduction in open area over time. It can result from **migration**, the movement of cells into the gap, and **proliferation**, an increase in cell number through division. A smaller gap alone does not prove faster migration. Compare each location with its starting gap and use the same imaging positions and magnification. Compare replicate wells, which are separate wells receiving the same treatment. Cell counts or **viability measurements**, checks of how many cells remain alive, help show whether growth or cell loss affected closure.

NHDFs may receive a treatment related to the NAMPT or hTERT experiments. One example is a tested **mesenchymal stromal cell extracellular vesicle (MSC EV) preparation**, a sample containing small membrane-bound particles released by MSCs. Record the treatment identity, amount, and exposure time. An untreated control shows closure without treatment. A **vehicle control** tests the liquid used to deliver the treatment. A preparation control helps test background material from sample preparation. Use matching culture and imaging conditions across groups.

Quality assurance (QA) includes steps that keep the process consistent and easy to track, such as labeling samples and following the written procedure. **Quality control (QC)** uses tests and controls to check whether samples and results meet stated criteria. **Mycoplasma** are small bacteria that can contaminate cultures without obvious cloudiness. They can change cell behavior and weaken treatment comparisons. Interpret mycoplasma tests only when their positive and negative controls work correctly.

Endotoxin is a component of the outer membrane of certain bacteria that can affect cell responses. It may remain even when living bacteria are not detected. An endotoxin assay needs its own controls and a check for **sample interference**, an effect of the sample that alters the test result. A **microbial-growth check** looks for organisms that grow under the chosen conditions. It may miss organisms that do not grow on the selected medium or during the observation period. A negative result alone does not prove that a sample is sterile or suitable for injection. Interpret all QC results alongside gap closure and cell viability.

MATERIALS

- NHDFs plated in a tissue-culture-treated 24-well plate with a nearly confluent monolayer
- Complete NHDF medium and assigned treatments and controls
- Sterile pipette tips for a scratch or validated gap-creating inserts
- PBS and fresh medium as directed by the class protocol
- Microscope with camera, consistent imaging positions, and image-analysis software
- Cell viability assay or cell counts
- MycAway Plus-Color kit 40612ES60 and its current instructions, if used
- An identified endotoxin assay kit and endotoxin-free supplies, if used
- Tryptic soy agar and any additional approved microbial-growth media, if used
- Appropriate positive and negative controls, labels, and laboratory record sheet

METHODS

Part A: wound scratch assay

1. Confirm that each NHDF well contains a healthy, nearly confluent monolayer. Record the cell passage, treatment assignment, and initial cell shape and appearance. Include untreated and appropriate vehicle or EV-preparation controls.
2. Create one gap per well using the class-approved tip or insert method. Keep the technique and approximate gap size consistent across wells.
3. Gently remove detached cells as directed by the class protocol, replace with the assigned medium or treatment, and begin timing. Avoid disturbing the remaining monolayer.
4. Image the same marked positions immediately after the gap is made (time zero) and at the planned later time points. Keep magnification and imaging settings consistent.
5. Measure the gap area or width from the images using one consistent analysis method. Record cell viability or growth at the same time so changes in closure can be interpreted.
6. For each measured location, calculate percent gap closure as $[(\text{initial gap area} - \text{later gap area}) \div \text{initial gap area}] \times 100$. Report replicate values and their variation.

Part B: research quality checks

1. For mycoplasma testing, collect the sample required by the MycAway Plus-Color kit. Include its positive and negative controls. Use the kit's reaction volumes and constant-temperature program. This kit uses a color-based test at a constant temperature, rather than a standard 94 °C PCR program. Read the colors using the kit instructions only when the controls are valid.
2. For endotoxin testing, identify the method used by the available kit. A gel-clot test detects clot formation. A chromogenic test measures color, and a turbidimetric test measures cloudiness. Follow the exact kit instructions for dilution, controls, timing, and interpretation. Do not substitute a light-absorbance reading for a gel-clot result. Include the required positive product control to check whether the sample interferes with the test.
3. For a classroom microbial-growth check, label approved agar plates and apply a measured aliquot of sample using the instructor's method and appropriate controls. Incubate and inspect according to the course's approved conditions and containment practices. Record growth or no observed growth during the specified observation period. Do not describe a negative plate as proof of sterility.

EXERCISE NHDF Wound Scratch Assay and Quality Checks

NAME _____

EXPECTATIONS

1. Predict how the gap area will change in treated and control wells if the treatment promotes NHDF gap closure. What other observation would help distinguish migration from increased proliferation?
2. Predict how a positive mycoplasma result in the source culture would affect the reliability of a treatment comparison. What result would you expect from a valid negative control?

RESULTS

Present representative time-zero and follow-up images, gap measurements, percent closure for each replicate, treatment conditions, and cell viability. Record the identity and result of each QC assay, its controls, and any invalid or inconclusive result. State the test's detection limits or other relevant limitations when known.

Student name: _____ Partner: _____

Date: _____ Group: _____ Experiment ID: _____

Record units with every measurement. Use N/A for tests not performed. Add rows or attach raw data as needed.

Treatment and imaging plan

Well ID	Treatment and amount	Control type	Passage / confluence	Imaging position

Gap measurements

Well / position	Time (h)	Initial gap area	Later gap area	Gap closure (%)	Viability / growth

Quality check results

Test / kit ID	Sample ID	Positive control	Negative control	Sample result	Valid? / notes

Calculations

Gap closure (%) = (initial gap area – later gap area) ÷ initial gap area × 100. Use the same area units.

Observations, excluded data, and changes from the procedure

Attach the labeled scratch images. File name or attachment ID: _____

CONCLUSIONS

1. Did gap closure differ between treatment and control wells? Support the answer with measurements and replicate variation.

2. Could changes in proliferation or viability explain the difference? What further experiment would separate these effects?
3. Were the mycoplasma, endotoxin, and growth-check results valid based on their controls? Explain.
4. What do the QC results allow you to say about this research experiment, and what claims would require additional testing?

Attributions:

ibidi. Wound healing and migration assay workflow: <https://ibidi.com/content/281-experimental-workflow>

Yeasen. MycAway Plus-Color kit 40612 product information: <https://www.yeasenbio.com/fr/products/40612>

USP. Bacterial Endotoxins Test methods: <https://www.usp.org/harmonization-st...ial-endotoxins>

FDA. Limits of sterility testing: <https://www.fda.gov/regulatory-infor...ility-protocol>

17: siRNA Knockdown of hTERT in Mesenchymal Stromal Cells

Learning Objectives

- Describe how an siRNA guide strand directs an RNA-induced silencing complex to target mRNA.
- Design hTERT knockdown and matched control conditions for MSCs.
- Carry out a reagent-specific siRNA transfection and record culture conditions and reagent exposure.
- Evaluate hTERT mRNA reduction and distinguish target knockdown from transfection toxicity or off-target effects.

BACKGROUND

Gene expression is the use of genetic information to make RNA and, often, a protein. **Messenger RNA (mRNA)** carries instructions for making a protein. **Small interfering RNA (siRNA)** is a short, double-stranded RNA used to reduce a matching mRNA. This reduction is called **knockdown**. Knockdown lowers gene expression; it does not permanently remove the gene.

After siRNA enters a cell, one strand acts as a **guide strand**, the strand that recognizes matching mRNA. It joins the **RNA-induced silencing complex (RISC)**, a group of proteins that uses guide RNA to find matching RNA sequences. An **Argonaute protein**, a protein in RISC, can cut the matching mRNA. The cell then has less mRNA available to make the protein. The effect is usually temporary. Existing protein may remain after its mRNA falls, so protein levels may change later.

Human telomerase reverse transcriptase (hTERT) is the catalytic protein component of **telomerase**, an enzyme complex that maintains sequences at chromosome ends. This experiment uses **mesenchymal stromal cells (MSCs)**, cultured cells with characteristic growth and differentiation properties. It tests the effects of lowering hTERT expression. First check that hTERT mRNA can be measured in the assigned cells. Some primary MSC samples may have levels too low for reliable analysis. If the cells were engineered to express hTERT, record the added sequence and check that the siRNA matches its mRNA.

Transfection delivers nucleic acids into cells. A lipid reagent contains fat-like molecules that help carry siRNA. The siRNA and reagent form particles before being added to cells. RNA amount, reagent amount, culture conditions, and collection time affect delivery and cell health. Follow the approved instructions for the exact reagent and cells. Do not assume that every procedure requires the same washing, exposure time, or serum-free treatment. Record the amounts and final concentration.

A **non-targeting siRNA** is a control RNA designed not to match the intended target. It is delivered with the same reagent and helps identify changes unrelated to hTERT targeting. A **reagent-only control** tests the delivery reagent without siRNA. A **positive-control siRNA** is known to reduce a control gene expressed in the cells. It can help show whether delivery and measurement work. Test two different hTERT siRNAs in separate wells. Agreement supports the interpretation, since one siRNA may also cause an **off-target effect**, an unintended change involving another RNA or a different treatment response.

Reverse transcription quantitative PCR (RT-qPCR) copies RNA into DNA and measures a selected sequence. Compare hTERT with a suitable **reference gene**, a gene whose measured RNA stays stable under the tested conditions. This adjustment is called **normalization**. Compare the adjusted results with the non-targeting control. The $2^{-(\Delta\Delta Ct)}$ method estimates relative expression when its requirements are met, including similar target and reference amplification efficiencies near 100 percent. Record cell shape and appearance and measure **viability**, the proportion or number of living cells, at the same time. Cell loss can distort the result or suggest toxicity. A fall in hTERT mRNA does not prove a fall in protein or telomerase activity; those require separate tests.

MATERIALS

- Human MSCs with confirmed measurable hTERT expression and cell-source information
- Approved 6-well plate plan and MSC growth medium appropriate for transfection
- Two distinct hTERT siRNAs, non-targeting siRNA, and validated positive-control siRNA if assigned
- Lipid-based siRNA transfection reagent and manufacturer's current 6-well protocol

- Reduced-serum complex-formation medium and sterile RNase-free tubes and tips
- Class II biosafety cabinet and required personal protective equipment
- Microscope and cell-viability assay specified by the instructor
- RNA extraction, reverse transcription, and RT-qPCR reagents with hTERT and reference-gene assays
- Culture and reagent-lot documentation forms

METHODS

1. Confirm the assigned MSC preparation has detectable hTERT mRNA and that each hTERT siRNA matches its intended transcript. Review the approved transfection protocol, reagent ratios, final medium volume, medium-change instructions, and planned mRNA and viability sampling times.
2. Prepare a plate map with separate wells for non-targeting siRNA, hTERT siRNA 1, hTERT siRNA 2, and reagent-only control. Include the validated delivery control and replicate wells if assigned. Use the same amount of delivery reagent in comparable siRNA conditions.
3. Seed healthy MSCs so they reach the confluence specified by the reagent protocol at transfection. Record cell source, passage, seeding number, plate map, medium lot, date, and operator. Inspect cell shape and appearance and confirm that the cultures are free of suspected contamination.
4. Prepare the biosafety cabinet and label RNase-free tubes for every condition. Prepare separate siRNA and lipid dilutions with the specified reduced-serum medium and volumes. Calculate the final siRNA concentration in each well from the amount of siRNA and the final well volume; do not assume that a given picomole amount produces the same concentration in every format.
5. Combine diluted siRNA and lipid in the order and ratio required by the current reagent protocol. Mix gently and allow complexes to form for the specified time. Prepare the reagent-only condition at the same time without siRNA. Keep each condition separate to avoid cross-contamination.
6. Add complexes to the appropriate wells containing the specified medium. Use serum-containing culture medium if allowed by the reagent protocol; omit antibiotics unless this combination has been validated. Do not routinely wash cells with PBS or replace medium after 6 hours unless the assigned MSC protocol requires it.
7. Gently distribute the complexes and return the plate to the specified incubator. Record actual siRNA and reagent volumes, lots, final well volume, complex-formation time, addition time, operator, and any change from the planned procedure.
8. Observe cell shape and appearance and viability at the assigned times. Change medium only if the validated protocol directs it. Record any detachment, rounded cells, or other sign of toxicity in each condition.
9. At the assigned time, collect cells separately from each well for RNA extraction, preserving the plate map and sample identities. Perform reverse transcription and RT-qPCR for hTERT and the validated reference gene. If protein analysis is assigned, collect separate samples at its specified time.
10. Calculate normalized hTERT expression relative to the non-targeting siRNA condition and report the percent remaining and percent knockdown for each hTERT siRNA. Compare both target siRNAs with the reagent-only, non-targeting, and delivery controls and with viability observations before interpreting the result.

EXERCISE siRNA Knockdown of hTERT in Mesenchymal Stromal Cells

NAME _____

EXPECTATIONS

1. Predict the hTERT mRNA results for each of the two hTERT siRNAs compared with the non-targeting control if both produce effective knockdown.
2. Predict what you might observe in the hTERT mRNA and viability measurements if a transfection condition is toxic. How would that affect your expected interpretation?

RESULTS

Record the plate map, MSC source and passage, siRNA identities and lots, reagent and medium lots, final siRNA concentrations, complex-formation and treatment times, cell shape and appearance and viability, and hTERT and reference-gene qPCR results. Show normalization and percent-knockdown calculations for each siRNA. Report missing or undetectable baseline expression and any changes from the procedure rather than assigning a numerical knockdown to an unreliable result.

Student name: _____ Partner: _____

Date: _____ Group: _____ Experiment ID: _____

Record units with every measurement. Use N/A for tests not performed. Add rows or attach raw data as needed.

MSC source/passage: _____ Reference gene: _____ RNA/reagent lots: _____

Complex formation time: _____ Addition time: _____ Medium/lot: _____

Culture and transfection record

Well / sample ID	Treatment / siRNA ID	mRNA sampling time	Viability (%)	Cell observations

Reagent preparation

Condition	siRNA amount and units	Lipid volume (μL)	Final well volume (mL)	Final siRNA conc. and units

qPCR measurements

Sample / replicate	hTERT C _q (Ct)	Reference C _q (Ct)	ΔC_q	Control / QC notes

Knockdown calculations

Sample / condition	$\Delta\Delta\text{C}_q$	Relative expression	Expression remaining (%)	Knockdown (%)

Calculations

$\Delta Cq = \text{target} - \text{reference}$. $\Delta\Delta Cq = \text{treated } \Delta Cq - \text{control } \Delta Cq$. Relative expression = $2^{(-\Delta\Delta Cq)}$ when assay requirements are met. Remaining (%) = relative expression $\times 100$. Knockdown (%) = $100 - \text{remaining} (\%)$.

Observations, excluded data, and changes from the procedure

CONCLUSIONS

1. Did each hTERT siRNA reduce normalized hTERT mRNA relative to the non-targeting control? Show the values and explain any difference between siRNAs.
2. Do the reagent-only and delivery controls support a conclusion that the transfection worked without unacceptable toxicity? Explain.
3. What can the mRNA results establish, and what additional protein or telomerase measurement would be needed to support a claim about hTERT function?
4. If the two hTERT siRNAs produced different effects on growth or viability, what explanations would you test before linking the observed cell response to hTERT knockdown?

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18: Quality Checks for NHDF Scratch Assay Samples

Learning Objectives

- Distinguish quality assurance from quality control and connect each to reliable cell culture results.
- Explain why mycoplasma, endotoxin, and microbial growth require different tests.
- Use assay controls to determine whether a test result is valid.
- Record results and explain the limits of a negative contamination test.

BACKGROUND

Use this exercise to evaluate cultures and cell-derived samples associated with an NHDF wound-scratch experiment involving NAMPT or hTERT. These checks support interpretation of the functional assay; they do not measure scratch closure themselves.

Normal human dermal fibroblasts (NHDFs) are connective tissue cells from human skin. A **wound-scratch assay** follows closure of a gap made in a cell layer. Both cell movement and cell division can contribute to closure. **Nicotinamide phosphoribosyltransferase (NAMPT)** participates in cellular metabolism, while **human telomerase reverse transcriptase (hTERT)** is a component of telomerase. Changes in closure after a treatment require suitable controls and comparable starting cultures. Contamination can alter cell behavior and obscure the treatment effect.

Quality assurance (QA) includes the procedures that prevent errors: approved instructions, staff training, equipment checks, sample labels, and complete records. **Quality control (QC)** consists of measurements used to evaluate a culture or sample. For example, confirming that an incubator was checked is a QA activity; testing a culture for mycoplasma is a QC activity. Neither activity alone establishes that a cell-derived preparation is suitable for a particular application.

Mycoplasma are small bacteria without cell walls. Contaminated cultures can look normal while their metabolism and gene expression change. The Yeasen test detects mycoplasma DNA using **loop-mediated isothermal amplification (LAMP)**, which increases the amount of target DNA at a constant temperature. A **positive control** confirms that the reaction can detect its target; a **negative control** checks for unintended contamination. Interpret samples only when both controls behave as expected.

Endotoxin is associated with **lipopolysaccharide (LPS)** in the outer membrane of Gram-negative bacteria. It can remain after bacteria die, so a plate with no growth does not exclude endotoxin. **Limulus amoebocyte lysate (LAL)** reagents respond to endotoxin. A **gel-clot assay** detects a firm clot at a stated sensitivity, whereas a **chromogenic assay** measures a color reaction using a separate procedure. These formats cannot share a single readout or calculation.

An agar screen detects organisms able to grow on the selected medium under the selected conditions. A negative result means no growth was detected in the tested portion; it does not prove complete sterility. **Extracellular vesicles (EVs)** are particles released by cells. An EV-enriched preparation, conditioned medium, and a source culture may require different sample preparation and interference checks.

MATERIALS

- Laboratory coat, gloves, eye protection, approved biosafety cabinet, labeled samples, and biohazard waste containers.
- Yeasen MycAway Plus-Color One-Step Mycoplasma Detection Kit 40612ES60 with its current instructions; capped reaction tubes; micropipettes suitable for 1–20 µL; filtered tips; tube rack; and temperature-controlled instrument capable of the required holds.
- Identified gel-clot LAL or TAL kit with instructions and lot certificate; certified endotoxin-free tubes, tips, water, and standards; timer; thermometer; and temperature-controlled incubator or water bath.
- 100 mm tryptic soy agar (TSA) plates, sterile spreader, P200 micropipette and sterile tips, labels, and instructor-approved microbiology incubator.
- Laboratory record sheet and camera or imaging device for documenting closed tubes and plates.

METHODS

A Preparation and documentation

1. Record sample ID, source culture, treatment, collection date, kit identity, lot numbers, expiration dates, and equipment IDs. Match every sample to its source record.
2. Review the instructions supplied with each kit. Record approved settings and control acceptance criteria before starting. The endotoxin procedure below applies to gel-clot testing.
3. Prepare separate aliquots for each test. Use aseptic technique and endotoxin-free supplies where required. Keep amplification controls away from culture work and add positive control material last.

B Mycoplasma detection

The following setup follows Yeasen manual Ver.EN20260429. Check that the supplied kit instructions match this revision.

1. Collect culture supernatant at the sampling interval specified by the kit and instructor. Record time since the last medium change or passage.
2. Mix kit reagents as instructed. Dispense 17 μL Color Lamp reagent and 2 μL primer reagent into each tube, including controls.
3. Add 1 μL culture supernatant to each sample tube and 1 μL supplied positive control to its tube. Leave the negative control without sample, as specified in this manual. Cap promptly.
4. Use instrument holds of 30 °C for 5 minutes followed by 63 °C for 60 minutes. For a water bath, use the manual's mineral oil overlay. Do not substitute conventional PCR cycling.
5. Cool to room temperature and inspect closed tubes against white paper. Record blue-purple as negative and sky-blue as positive only if controls pass. Flag ambiguous colors or failed controls for repeat testing.

Record actual reaction setup and temperatures. Keep amplified tubes closed and discard them through the approved laboratory waste procedure.

C Endotoxin testing by gel clot

The instructor must identify the kit and approve its operating instructions before testing. **Endotoxin units (EU)** express biological activity; kit sensitivity is reported in EU/mL. Record the kit threshold and dilution factor.

1. Using the kit instructions, prepare reagents and an endotoxin-free water negative control, an endotoxin standard positive control, test samples, and a spiked sample control. Use the specified number of replicates.
2. A **spiked sample control** contains sample plus a known endotoxin amount. It checks whether the sample interferes with clot formation. Prepare its spike concentration according to the kit instructions.
3. Dispense the sample and reagent volumes specified by the identified kit. Record any sample dilution. Do not infer concentrations from reagent labels such as R1–R4.
4. Incubate without disturbance at the kit's specified temperature and duration. Record actual conditions; do not use a universal 38 °C or one-hour setting.
5. At the specified endpoint, read clot formation using the kit's handling method. Record each tube as positive, negative, or inconclusive. Do not measure gel-clot samples at 395 nm or construct an absorbance standard curve.
6. Interpret sample results only when the negative, positive, and interference controls meet kit criteria. A valid negative is below the test threshold under the conditions used, rather than proof of zero endotoxin.

If a chromogenic kit is supplied instead, use its complete manufacturer procedure, including substrate, wavelength, standards, timing, and interference controls. Do not use this gel-clot procedure for quantitative color measurements.

D Agar contamination screen

1. Prepare the biosafety cabinet according to the laboratory SOP. Label the plate underside with sample ID, date, and initials. Include an uninoculated medium control and a handling control prepared with sterile diluent.

2. Pipette 100 μ L sample onto TSA and spread evenly with a sterile spreader. Use a new tip and spreader for each sample. Replace the lid promptly.
3. Incubate plates inverted under instructor-approved conditions suitable for the organisms being screened. Record temperature and duration. A separate fungal screen requires its own medium and conditions.
4. Observe plates while closed at 48 hours and at any additional approved endpoint. Record growth, colony appearance, and control results. Photograph the closed plate if requested.
5. Report growth to the instructor and keep suspect cultures separate. Dispose of plates through the approved decontamination and biohazard waste procedure.

TSA incubation for 48 hours is a limited screen. Organisms that grow slowly, require other conditions, or are absent from the tested aliquot may be missed.

EXERCISE Quality Checks for NHDF Scratch Assay Samples NAME _____

EXPECTATIONS

1. If a culture contains mycoplasma but looks healthy, what result do you predict for the DNA test and why?
2. Could an agar screen show no growth while an endotoxin test is positive? Explain your prediction.

RESULTS

Sample ID and treatment: _____ Collection date: _____

Test	Controls and validity	Sample observation	Interpretation
Mycoplasma	PC: ____ NC: ____ Valid: ____	Color: _____	_____
Gel-clot endotoxin	Positive: ____ Negative: ____ Spike: ____ Valid: ____	Clot: _____ Dilution: _____	Threshold: ____ EU/mL _____
TSA screen	Medium: ____ Handling: ____ Valid: ____	Growth: _____ Time: ____ Temp: ____	_____

Kit lot and manual revision: _____

Actual test settings and deviations: _____

Disposition and instructor review: _____

CONCLUSIONS

1. Identify one QA activity and one QC result from this exercise. How did each contribute to confidence in your data?
2. Explain how mycoplasma, endotoxin, or microbial growth could complicate interpretation of NHDF scratch closure after a NAMPT or hTERT treatment.
3. If a control fails, why must the associated sample result be withheld? Use either a failed mycoplasma control or a failed endotoxin spike as your example.

4. What can you conclude from negative results in all three tests, and what remains uncertain? Describe the next action if any test is positive or inconclusive.

Attribution:

Yeadon. MycAway Plus-Color One-Step Mycoplasma Detection Kit manual Ver.EN20260429. Product and manual: <https://www.yeadonbio.com/products/40612>

Charles River. LAL Reagents for LAL Assays. <https://www.criver.com/products-services-accessories>

Use the exact endotoxin kit instructions and lot certificate as the operating reference for Part C.

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TEMPLATE

Learning Objectives

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EXPECTATIONS

RESULTS

CONCLUSIONS

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biology

Tissue Culture Lab Manual (Biot 77 IVC)

Biotechnology

Tissue Culture Lab Manual (Biot 77 IVC)

C _____

cell culture

Tissue Culture Lab Manual (Biot 77 IVC)

I _____

Irvine Valley College

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T _____

tissue culture

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GLOSSARY

S

Sample Word 1 | Sample Definition 1

DETAILED LICENSING

Overview

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