How to Passage Cell Culture: Standard Operating Procedure

This SOP describes how to passage cell culture for both adherent and suspension mammalian cell lines, covering monitoring of the growth curve, sterile dissociation of adherent monolayers, single-cell suspension preparation, cell counting, reseeding, and routine culture maintenance. Following this procedure consistently supports healthy cell yield and reproducible experimental results.

Purpose

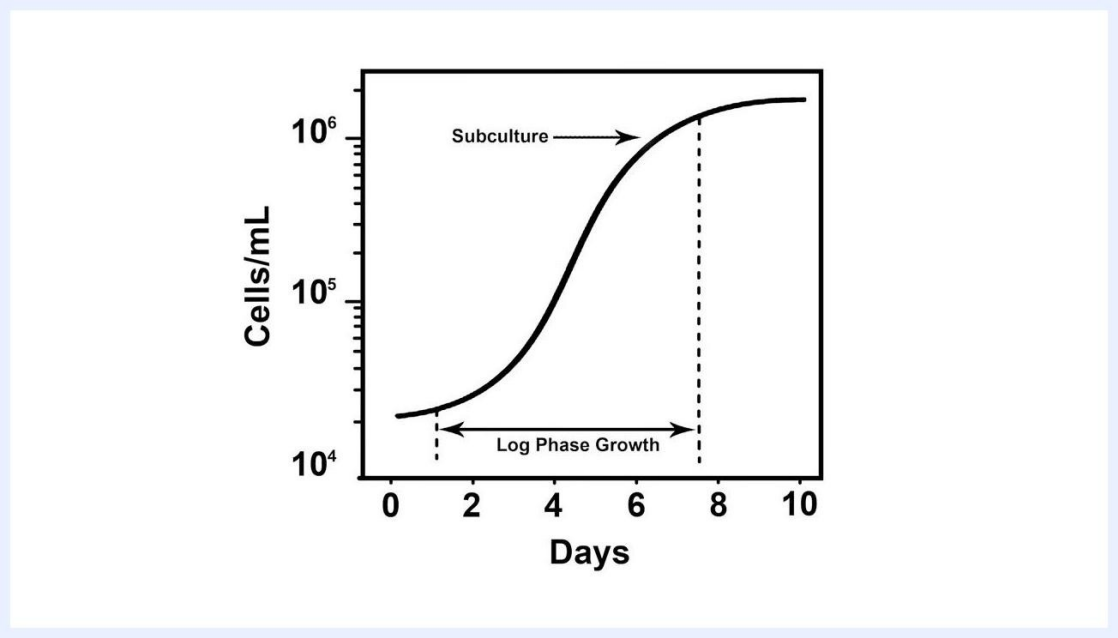
This method defines the standard technique for how to passage cell culture, including subculturing adherent monolayers and suspension cultures, counting cells, and reseeding fresh flasks at an appropriate density. The intended use is routine maintenance of mammalian cell lines to sustain cells in their optimal growth phase for downstream experimental use.

Scope

This procedure applies to adherent and suspension mammalian cell cultures grown in standard culture flasks. It covers work performed inside a biosafety cabinet using a centrifuge, a microscope or automated cell counter, and a CO_2 incubator. It is intended for trained laboratory personnel following institutional biosafety and cell culture protocols. This SOP does not define cell-line-specific seeding densities, dissociation reagent concentrations, or centrifugation speeds and durations, which must be obtained from the relevant cell-line protocol.

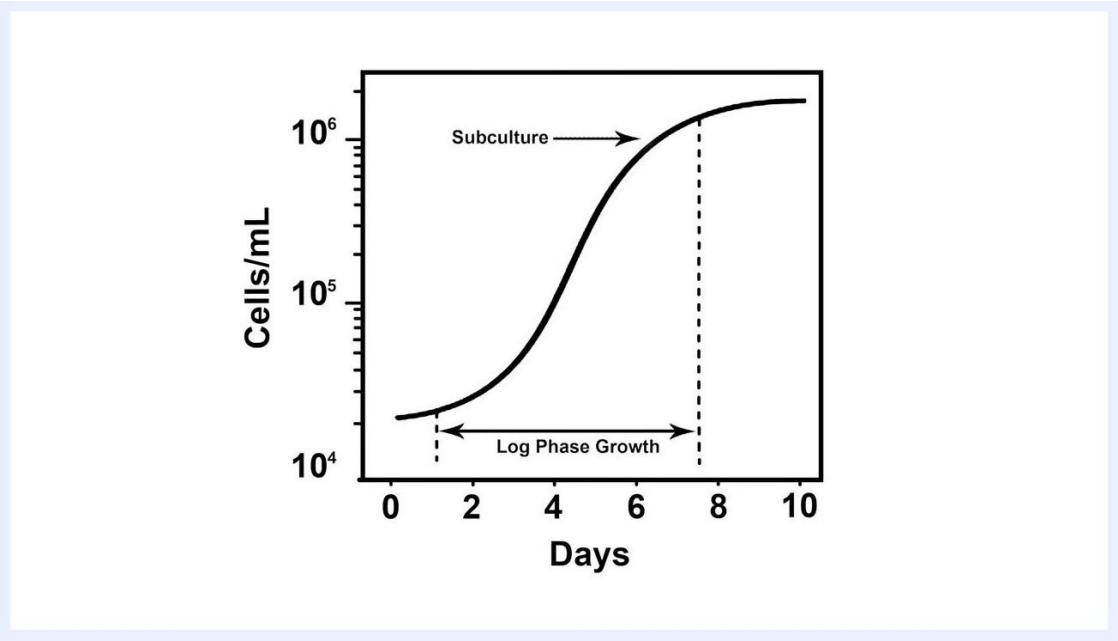
Principle

After seeding, cells enter a period of rapid, exponential division known as the log phase. The log phase is critical for maximizing healthy cell yield and typically occurs between days 2 and 7 after seeding, as shown on the growth curve below.



Growth curve showing the log phase between days 2 and 7, with the y-axis labeled "Cells/mL" (10^4 to 10^6) and the x-axis labeled "Days" (0 to 10)

Cultures should be maintained in the log phase to ensure the highest proportion of healthy cells for experiments. Cells are passaged (subcultured) when they cover the plate or when cell density exceeds the medium's capacity, with the optimal time being at the end of the log phase, before the culture reaches the plateau phase.



Full cell growth curve labeled "Log Phase Growth" and "Subculture," with the y-axis labeled "Cells/mL" and the x-axis labeled "Days"

The method relies on enzymatic or non-enzymatic dissociation reagents to detach adherent cells from the culture surface, producing a single-cell suspension. Viability is assessed using Trypan Blue exclusion, which distinguishes live cells (intact membranes, appearing white or colorless) from dead cells (which take up the blue stain).

Materials and Reagents

Category	Item
Equipment	Biosafety cabinet (e.g., HERAsafe KS)
Equipment	Centrifuge
Equipment	Microscope or automated cell counter
Equipment	CO_2 incubator
Consumables	Sterile pipettes and pipette controller
Consumables	Conical centrifuge tubes
Consumables	Culture flasks with vented or non-vented caps

Consumables	Tube racks
Reagents	Balanced salt solution without calcium and magnesium (e.g., DPBS)
Reagents	Cell dissociation reagent (e.g., trypsin or a gentle alternative such as Triple Express)
Reagents	Trypsin inhibitor (for serum-free conditions) or collection medium containing serum
Reagents	Complete growth medium (warm)
Reagents	Trypan Blue stain
PPE	Gloves and lab coat

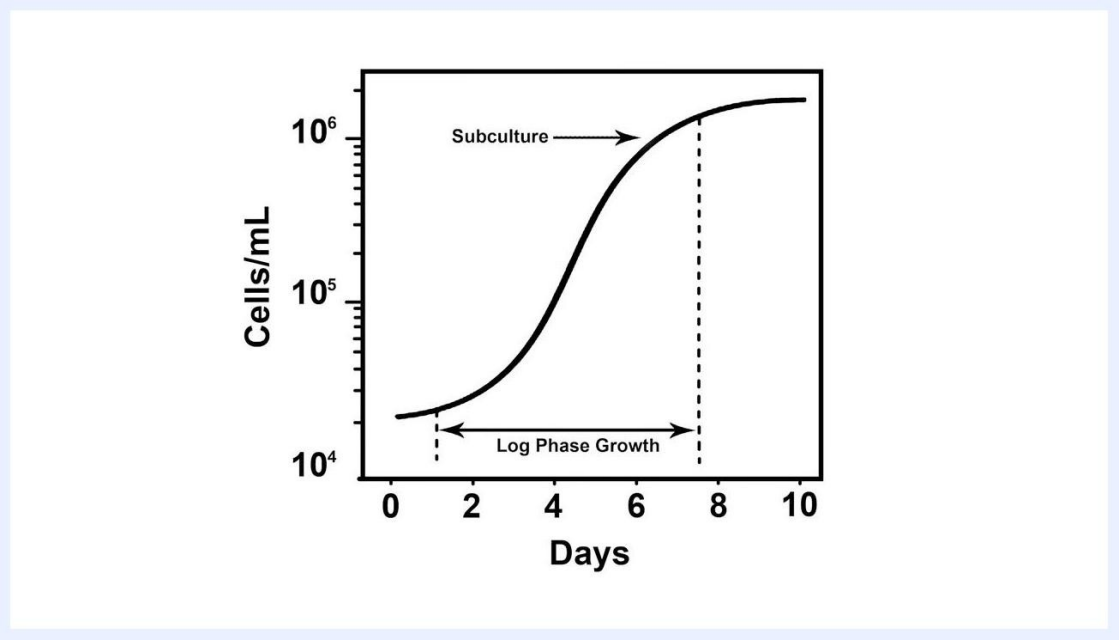
Ensure the balanced salt solution used for rinsing does not contain calcium or magnesium, as these ions can inhibit the action of cell dissociation reagents. Warm the complete growth medium before use.

Procedure

Preparing for cell culture work

1. Sterilize the biosafety hood and gather all required supplies before retrieving flasks from the incubator, including sterile pipettes, balanced salt solution, cell dissociation reagent, and culture media.

2. Organize the workspace so all materials are within reach to maintain sterility throughout the procedure.

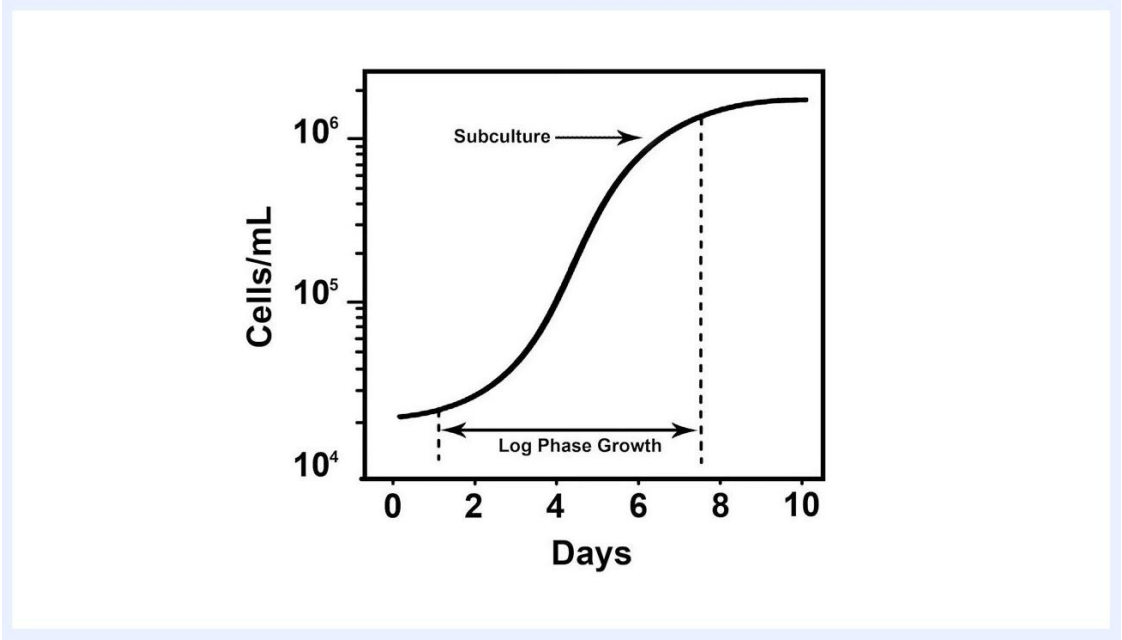


Person in lab coat and gloves working at a HERAsafe KS biosafety cabinet, with supplies and equipment arranged inside the hood

Dissociating adherent cells

3. Examine the cell culture for signs of contamination or deterioration before proceeding.
4. Handle the flask gently during transport to avoid disturbing the monolayer.

5. Remove the spent medium from the flask using a sterile pipette and pipette controller.



Gloved hands preparing to remove medium from a cell culture flask, with bottles of media and reagents on the work surface

6. Rinse the cells with a balanced salt solution such as DPBS to remove residual medium and serum.

Gloved hands using a pipette to add solution to a cell culture flask, with labeled bottles and a green tube rack visible

7. Promptly remove the salt solution from the flask, working efficiently to minimize the time cells are exposed to air without liquid.

Gloved hands holding a capped cell culture flask after removing the salt solution

8. Immediately add the next solution each time liquid is aspirated from the cells to prevent drying.

Gloved hands using a pipette to add solution to a cell culture flask, with bottles of media and reagents nearby

9. Add just enough cell dissociation reagent to cover the cell sheet to detach the cells from the plate.

Gloved hands adding dissociation reagent to a cell culture flask using a pipette, with labeled bottles and equipment visible

10. Confirm that the dissociation solution completely covers the cells and gently tap the side of the flask to help cells detach.

Gloved hands holding a cell culture flask with a blue cap inside a biosafety cabinet, surrounded by bottles of media and reagents, with tube racks visible

11. Use a microscope to confirm that cells have detached; detached cells appear round and move or slide when the flask is tilted.

Cell culture flask with a blue cap resting on the work surface inside a biosafety cabinet, with no hands visible

12. Avoid leaving the dissociation reagent on the cells for too long, especially with reagents other than gentle options like Triple Express, as overexposure can cause damage. If clumps remain, break them up by pipetting warm medium over them repeatedly to achieve a single cell suspension.

Gloved hands using a pipette controller to aspirate or dispense liquid into a cell culture flask, with bottles of media and reagents nearby

13. If using trypsin, inactivate it by adding collection medium containing serum, or use a trypsin inhibitor under serum-free conditions; some reagents are inactivated by dilution alone.

Gloved hands holding a pipette controller and a cell culture flask, preparing to add or remove liquid, with bottles of media and reagents visible

14. Transfer the cell suspension to a conical centrifuge tube and centrifuge to pellet the cells and remove residual dissociation reagent, using a speed and duration appropriate to the cell type.

Gloved hands transferring cell suspension into a conical tube using a pipette controller, with a green tube rack and bottles of media visible

15. Confirm a well-formed cell pellet at the bottom of the tube, then carefully remove the supernatant without disturbing the pellet and discard it into a designated waste container.

Gloved hands holding a pipette controller and a conical tube with a visible cell pellet at the bottom, surrounded by bottles of media and reagents

16. Resuspend the cell pellet in warm, complete growth medium using gentle pipetting to disperse the cells into a homogeneous single cell suspension.

Person in lab coat and gloves gently pipetting to resuspend cells in a conical tube inside a biosafety cabinet, with bottles of media and tube racks visible

17. Remove a small sample of the suspension for cell counting using your preferred method.

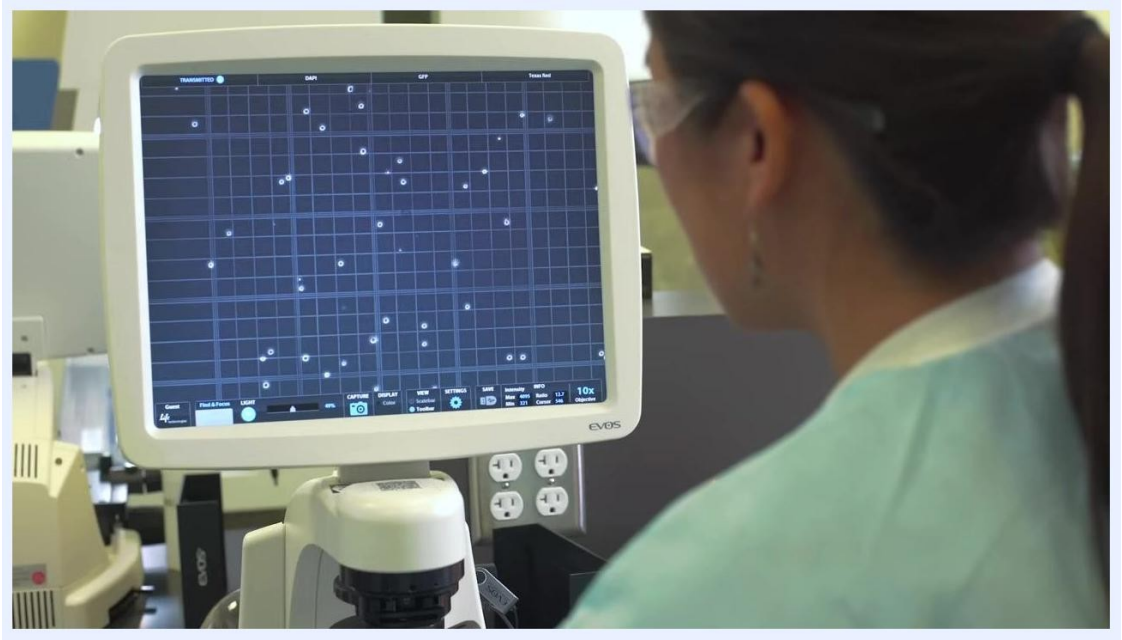
Counting cells

18. Stain a sample of the cell suspension with Trypan Blue to distinguish live cells (white or colorless) from dead cells (which absorb the blue stain).

19. Mix the suspension gently with a pipette to ensure a homogeneous single-cell solution before counting.

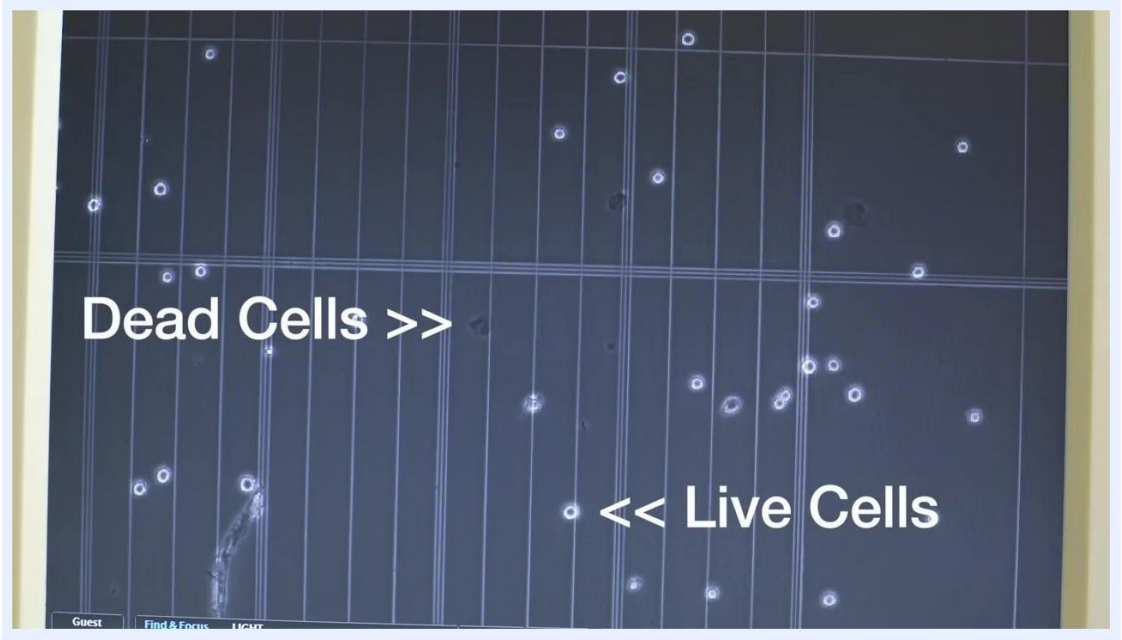
Person in lab coat and gloves gently pipetting to mix cell suspension in a conical tube inside a biosafety cabinet, with bottles of media and tube racks visible

20. Load the stained suspension onto a hemocytometer or into an automated cell counter and observe under a microscope or the counter's display.



Scientist in lab coat and safety glasses viewing a microscope display screen showing a grid with cells for counting

21. Identify and count the total number of cells and the number of dead (blue) cells using the grid.



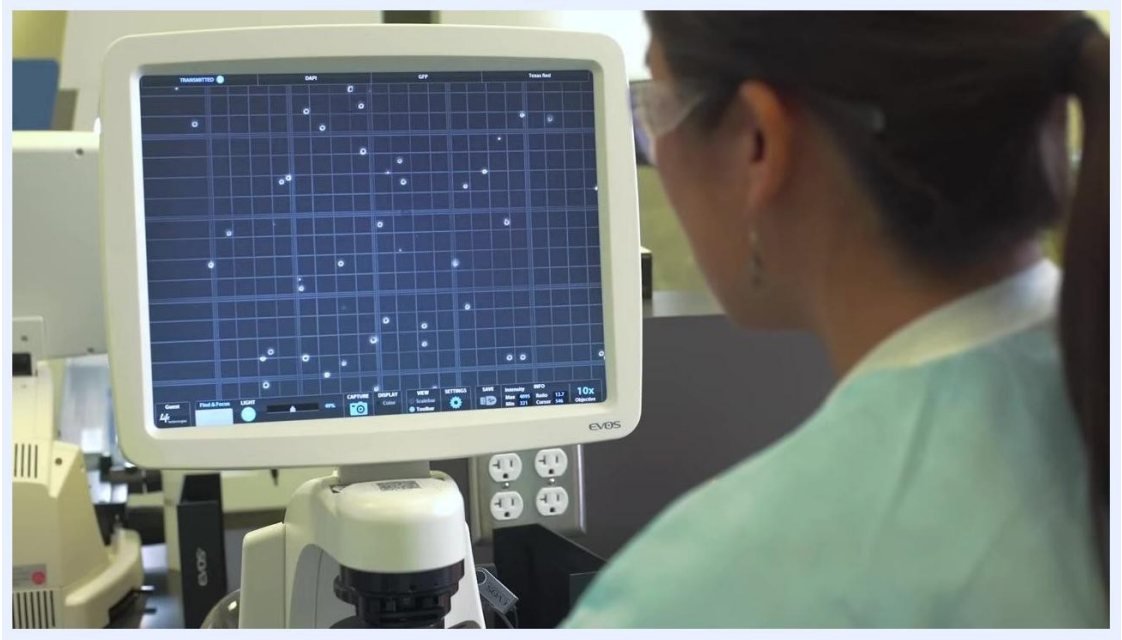
Close-up of a microscope display screen labeled "Dead Cells >>" and "<< Live Cells" with cells visible on a grid

22. Record the live and dead cell counts and calculate the percentage of live and dead cells.

Seeding new adherent cultures

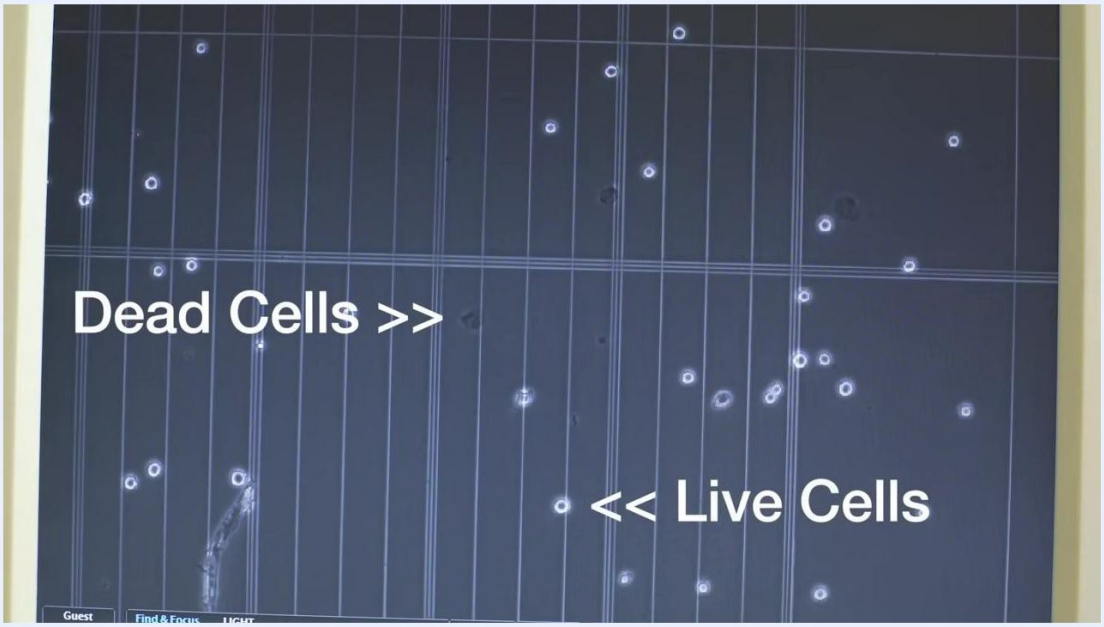
23. Determine the volume of fresh medium needed to reach the recommended seeding density from your protocol, based on the cell count.

24. Add the calculated volume of fresh medium to the cell suspension, mix gently, and pipette the cell solution into fresh, sterile culture flasks.



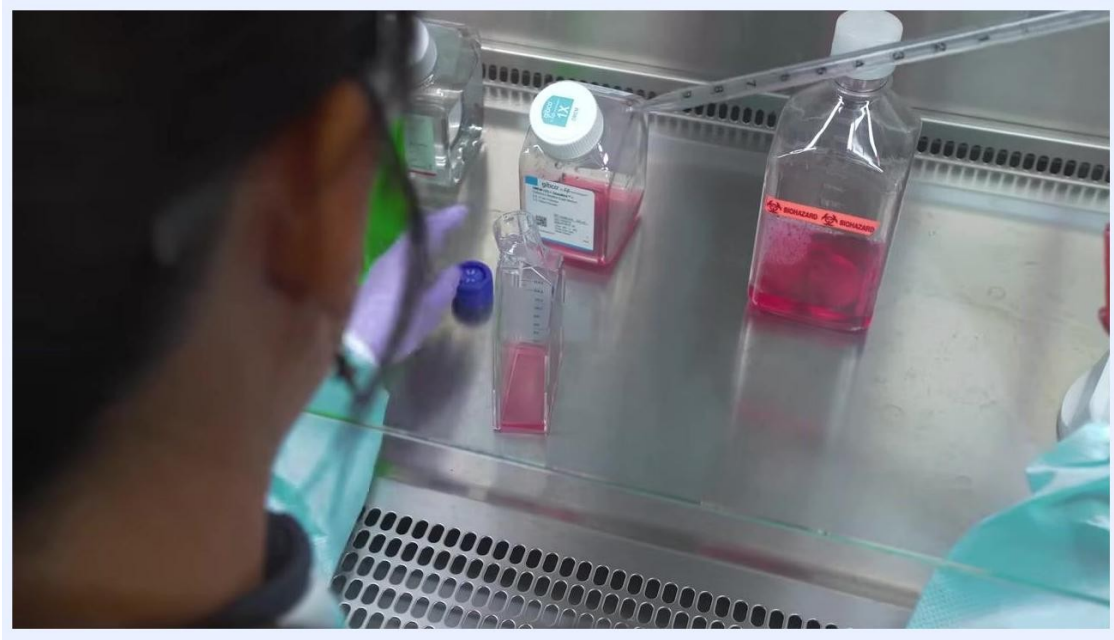
Gloved hands adding medium to a conical tube using a pipette controller, with bottles of media and tube racks visible

25. Cap the flasks appropriately: tighten vented caps securely, or leave non-vented caps slightly loose to allow gas exchange while preventing contamination.



Gloved hands capping a cell culture flask containing medium, with bottles of media and tube racks visible

26. Distribute the cells evenly by gently rocking the flask in a north-south-east-west motion.



Scientist gently rocking a capped cell culture flask to distribute cells evenly, with bottles of media and tube racks visible

27. Transport the flasks to the incubator and place them level on a shelf, ensuring they are not stacked directly on top of one another.

Incubator interior with multiple capped, labeled cell culture flasks containing red medium, arranged on shelves

Passaging suspension cells

28. Remove a small sample from the suspension cell culture flask using a sterile pipette for counting.

Gloved hands using a pipette controller to remove a sample from a flask for cell counting, with tube racks and media bottles visible

29. Count the cells using Trypan Blue and your preferred method, recording the total cell count and viability.

Gloved hand interacting with an automated cell counter touchscreen displaying total concentration and live/dead percentages

30. Add the appropriate volume of fresh medium to the flasks based on the cell count, staying within the minimum and maximum recommended volumes for the flask size to maintain proper air exchange and shaking flow.
31. Split the culture into multiple flasks if necessary to avoid overcrowding and maintain healthy growth.

32. Cap the flasks appropriately depending on whether they are vented or non-vented.

Gloved hands capping a flask after adding medium, with tube racks and media bottles visible in a biosafety cabinet

33. Return the flasks to the incubator, ensuring proper placement for optimal growth conditions.

Routine culture maintenance

34. Every three weeks, perform a medium change: gently centrifuge the cells to pellet them, carefully remove the old medium without disturbing the pellet, and replace it with completely fresh medium to remove accumulated cell debris and metabolic waste.

35. Return the culture flasks to the incubator, ensuring they are securely capped, placed level, and not overcrowded, and confirm the incubator is set to the correct temperature and CO_2 conditions for the cell line.

Gloved hands placing capped Erlenmeyer flasks with cell culture medium onto a shelf inside a Thermo-labeled laboratory incubator, alongside flasks already containing red medium

Calculations

Cell viability is calculated as the percentage of live cells relative to the total cell count obtained from the hemocytometer or automated cell counter (live cells appear white or colorless; dead cells appear blue after Trypan Blue staining). The required medium volume for reseeding is determined from the recorded cell count against the seeding density specified in your protocol. This SOP does not specify a numeric formula, dilution factor, or reporting units; these should be defined in the cell-line-specific protocol referenced during seeding density determination.

Quality Controls

Control Point	Requirement
Sterile technique	Perform all steps inside a biosafety cabinet with sterile pipettes and pipette controllers
PPE	Wear gloves and a lab coat during all handling steps

Waste disposal	Dispose of spent medium and dissociation reagents in designated biohazard waste containers
Culture monitoring	Inspect cultures for contamination or deterioration before each passage
Viability check	Confirm complete cell detachment under the microscope before proceeding
Documentation	Record medium change dates, cell counts, viability, and any observations on cell health or contamination

Scientist in lab coat and gloves using a pipette controller to transfer liquid into a test tube inside a biosafety cabinet, with a green tube rack, Erlenmeyer flasks, media bottles, and a biohazard-labeled flask visible

Accurate cell counting and proper seeding density are critical for reproducible results and healthy cultures. This SOP does not define numeric acceptance criteria or a failed-run protocol; if viability or seeding density falls outside expected ranges, consult your institution's cell culture protocol or laboratory supervisor before proceeding.

What's Next

Document all maintenance actions, including the date of medium change, cell counts, and observations regarding cell health or contamination. For further information or support, refer to your institution's protocols or contact your laboratory supervisor. For additional detailed protocols and troubleshooting guidance, consult the relevant technical support resources available to your laboratory.

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