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Protocol: Thawing and Seeding Mammalian or Insect Cells in Optimum Growth® Flasks

Purpose and Scope

General procedure for thawing cryopreserved mammalian or insect suspension cells into a 125mL or 250mL Thomson flask to a concentration of 0.5x10^6 cells/mL. Match post-thaw cell number to an appropriate flask working volume and shaker speed to maintain the desired seeding density and agitation environment during recovery.

Safety and Handling Requirements

- Perform all open handling in a certified biosafety cabinet using aseptic technique.
- Wear appropriate PPE and handle cryovials cautiously when retrieving from liquid nitrogen storage.
- Disinfect all items with 70% ethanol before use.

Materials and Equipment

- Cryovial containing mammalian or insect suspension cells
- Cell-line-appropriate complete growth medium, equilibrated to the temperature specified by the cell-line supplier.
- Sterile 125mL or 250ml Thompson flask, pre-labeled
- 37°C water bath or validated dry thawing device
- 70% ethanol, sterile wipes, serological pipettes, and sterile tips
- Cell counter and viability dye
- Appropriate shaker incubator for the cell type. Mammalian cultures are typically maintained at 37°C with CO_2 control, while insect cultures are commonly maintained at 27°C, without CO_2.

Procedure

Seeding Overview

Use the table below to determine the appropriate final culture volume, Thomson flask size, and fill volume based on the cryopreserved stock concentration and frozen stock volume. Clarify that the calculation assumes the frozen-stock concentration represents viable cells and that recovery is sufficient. If concentration is total cells, post-thaw viability must be considered when calculating the viable seeding density. while maintaining an appropriate flask fill volume for shaking culture. The taget seeding density calculation assumes a high viability frozen stock..

Thawing and Seeding Volume Table

Frozen Stock Concentration	Frozen Stock Volume	Final Seeding Density	Final Culture Volume	Recommended Optimum Growth® Flask	Shaking rpm (25mm orbit)
10x10^6 cells/mL	1mL	0.5x10^6 cells/mL	20mL	125mL-Standard PN 931110	120*
20x10^6 cells/mL	1mL	0.5x10^6 cells/mL	40mL	125mL-Standard PN 931110	140-150
50x10^6 cells/mL	1mL	0.5x10^6 cells/mL	100mL	250mL-Standard PN 931111	140-150

* Once cells have recovered from thaw and are doubling normally, they should be passaged to appropriate larger flask and speed should be increased to 140-150rpm

General Thaw and Seed Workflow

This protocol provides example calculations using a target seeding density of 0.5 × 10^6 cells/mL. Cell-line-specific seeding densities should take precedence.

1. Confirm the cryovial identity, stock concentration, passage, and required final seeding density before thawing.
2. Select the appropriate Thomson flask and final culture volume using the dilution table.
3. Pre-warm the required volume complete medium and add it to the labeled flask.
4. Thaw the cryovial rapidly until almost no ice remains, then disinfect the vial before transferring into the biosafety cabinet.
5. Aseptically transfer the thawed cell suspension into the flask using a sterile pipette and mix gently to distribute cells evenly without excessive shear.
6. Place the flask into the appropriate shaker incubator using validated cell-line-specific temperature, CO_2, orbit, and rpm settings.
7. Measure viable cell density and viability daily to ‘assess recovery

Recovery Monitoring Timepoints

Timepoint	Assessment / Documentation
Time 0	Record vial ID, passage, stock concentration and seeding density
24 hr	Assess viable cell density, viability, morphology, suspension behavior, and contamination status. A temporary lag in growth may occur during post-thaw recovery.
48–72 hr	Confirm improving growth trend, suspension uniformity, and recovery toward the expected cell-line-specific growth rate. Determine readiness for passage, scale-up, or downstream use according to established acceptance criteria.

Troubleshooting

Observation	Possible Cause	Action
Low viability	Slow thaw, prolonged DMSO exposure, or harsh handling, suboptimal cryopreservation.	Thaw rapidly, minimize exposure to cryoprotectant-containing freezing medium, according to the cell-line supplier’s protocol, and handle gently. Evaluate other vials from same freeze down.
Non-uniform suspension	Incorrect volume, density, orbit/RPM, or insufficient mixing	Confirm target volume, target density, and validated agitation settings.
Clumping or debris	Cell damage, DNA release, or poor dilution	Avoid vortexing, and follow cell-line-approved recovery steps.