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Thawing and Seeding Frozen Cells

Version 2

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Kenneth Schackart[1]

[1]University of Arizona

Kenneth Schackart

ABSTRACT

How to thaw cells from the liquid nitrogen storage and seed into a tissue culture flask

GUIDELINES

- Gloves must be worn at all times.
- Perform all tasks within biosafety cabinet.
- Anything entering biosafety cabinet must be generously sprayed with 70% ethanol (even you).
- When finished, wipe biosafety cabinet with 70% ethanol, and UV for at least 15 minutes.

MATERIALS TEXT

(1) T-75 or T-25 flask per frozen cell vial (or more if plating at a lower density)
(1) 15 mL centrifuge tube per frozen cell vial
(1) 10 mL serological pipet tip per T-75 flask or (1) 5 mL serological pipet tip per T-25 flask
Warmed cell culture media
1000 µL filter pipette tips

Thaw Cells

1 Thaw cells by suspending cryotube in **37 °C** water bath until completely thawed, but no longer than necessary

Transfer cell suspension

2 Within biosafety cabinet, transfer cell suspension to 15 mL centrifuge tube using 1000 µL pipette.

Dilute freezing medium

3 Add **1 ml** warmed cell culture medium to cell suspension *dropwise*.

Adding the inital cell culture medium slowly helps prevent cell death caused by a rapid change in osmotic pressure.

4 Add an additional **3 ml** warmed cell culture medium to cell suspension slowly.

Centrifuge cell suspension

5 Centrifuge the cell suspension at **1500 rpm** for **00:03:00** .

Resuspend Cells

6 Remove bulk of supernatant with serological pipet, then remove remainder with 1000 µL pipette.

For small cell pellets, you are better off leaving a small amount of media than disturbing the cell pellet.

7 Add **1 ml** warmed cell culture media to cell pellet.

Allowing the cell pellet rest in media for about 2 minutes will help with resuspension.

8 Gently pipette mix the cell pellet into the solution.

9 Add an additional **7 ml** warmed cell culture media [**3 ml** for T-25].

Seed Cells

10 Using a serological pipet, transfer the cell suspension to the tissue culture flask.

Label Flask

11 Label the flask with:
- Cell line
- Passage number
- Date
- Your intials

Incubate

12 Transfer flask to CO_2 incubator.

Documentation

13 Don't forget to remove the vial you used from the frozen storage inventory.