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 We use this protocol and it's working

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Thawing and Seeding Frozen Cells V.1

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ABSTRACT

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

GUIDELINES

Wear gloves. Spray anything entering or exiting the biosafety cabinet with 70% Ethanol.

MATERIALS

- (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 pipette tip per T-75 flask or (1) 5 mL serological pipette tip per T-25 flask
- Warmed cell culture media
- 1000 µL filter pipette tips

BEFORE START INSTRUCTIONS

UV appropriate number of flasks, 15 mL centrifuge tubes, waste beaker, and serological pipette tips.
 Warm appropriate cell culture media.

Thaw Cells

- 1 Thaw cells by suspending 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*.

Note

Adding the initial 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 1.5 kRPM for 00:03:00 .

Resuspend Cells

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

Note

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.

Note

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 if using a T-75 flask.
Add an additional 3 mL warmed cell culture media if using a T-25 flask.

Seed Cells

- 10 If using a T-75 flask, first add 2 mL warmed cell culture media to the flask.
Using a serological pipette, transfer the cell suspension to the tissue culture flask.

Label Flask

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

Incubate

12 Transfer flask to CO₂ incubator.

Documentation

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