Feb 26, 2019

Working

Thawing and Seeding Frozen Cells

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dx.doi.org/10.17504/protocols.io.xkmfku6

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ABSTRACT

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

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

GUIDELINES

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

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 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 STARTING

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*.

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

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.

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 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 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.