VERSION 2

JUN 05, 2019

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.3rcgm2w

Protocol Citation: Kenneth Schackart 2019. Thawing and Seeding Frozen Cells.

protocols.io
<https://dx.doi.org/10.17504/protocols.io.3rcgm2w>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
 We use this protocol and it's working

Created: Jun 04, 2019

Last Modified: Jun 05, 2019

PROTOCOL integer ID:
 24068

Thawing and Seeding Frozen Cells V.2

Kenneth Schackart¹

¹University of Arizona

Yoon Lab

Kenneth Schackart
 University of Arizona

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

(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

BEFORE START INSTRUCTIONS

- Warm cell culture media, DPBS, and Trypsin-EDTA in 37 °C water bath.
- Wash waste beaker with soap and warm water, then dry with paper towel.
- Expose serological pipet tips, centrifuge tube, and waste beaker to UV for at least

00:15:00

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*.

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 1500 rpm for 00:03:00 .

Resuspend Cells

- 6 Remove bulk of supernatant with serological pipet, 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 [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 initials

Incubate

12 Transfer flask to CO₂ incubator.

Documentation

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