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Version 2

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Thawing adherent cancer cell lines V.2

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1 Works for me This protocol is published without a DOI.

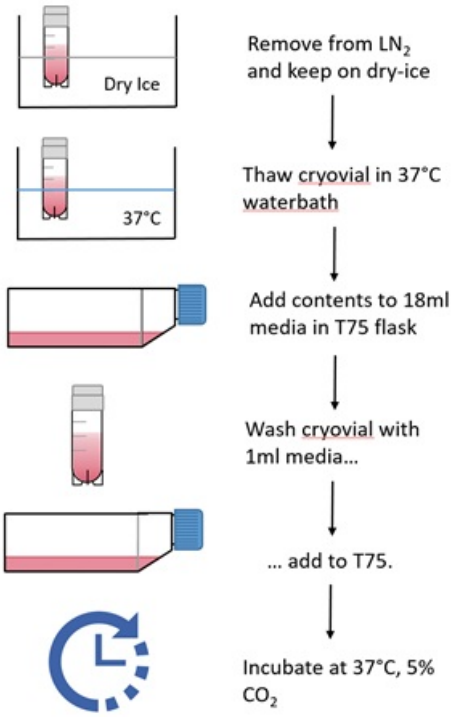
Cellular Generation and Phenotyping

Emily Souster

ABSTRACT

This SOP is for thawing an adherent cancer cell line from a frozen cryovial.

Process diagram:



PROTOCOL CITATION

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MATERIALS

NAME	CATALOG #	VENDOR
Cell culture treated T75 flasks	430641	Corning

MATERIALS TEXT

Select an appropriate culture media for your cell line. Common culture medias used for cancer cell lines are serum supplemented Advanced DMEM F-12 or RPMI, in the presence of pen-strep.

Equipment

Pipette boy
25ml stripette
P1000 pipette and tips
Microbiology Safety Cabinet (MSC)
Light Microscope
37 °C waterbath
37 °C , 5% CO_2 incubator

SAFETY WARNINGS

Retrieving vials from liquid nitrogen tanks is a significant ergonomic risk due to potential weight of filled racks. To reduce the risk of manual handling injury, lifting racks out of dewers should be kept to a minimum.

BEFORE STARTING

- Pre-warm complete culture media to room-temperature.
- Add 18ml complete culture media to a T75 flask.

1 Remove the cryovial from liquid nitrogen storage and place on dry-ice for transfer to cell culture lab.

2 Take the cryovial from the dry-ice and hold in a **37 °C** waterbath until thawed.

Be careful here to **avoid submerging** the lid or rim of the cryovial in the waterbath.

Dry the cryovial thoroughly, spray with 70% ethanol and transfer to cell culture hood.

3 Use a P1000 pipette to transfer the contents of the cryovial to the T75 flask containing **18 mL** media.

4 Use **1 mL** complete culture media to wash the cryovial and add this to the flask.

5 Place the flask in a **37 °C** , 5% CO_2 incubator. Agitate the flask gently back and forth and side to side to ensure even distribution of cells across the flask.

6 After 24 hours, inspect adherence and confluency. Remove the media using a sterile aspirating pipette and replace with **12 mL** complete culture media.