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## Freezing cancer cell lines

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Cellular Generation and ...



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**Protocol status:** Working

**We use this protocol and it's working**

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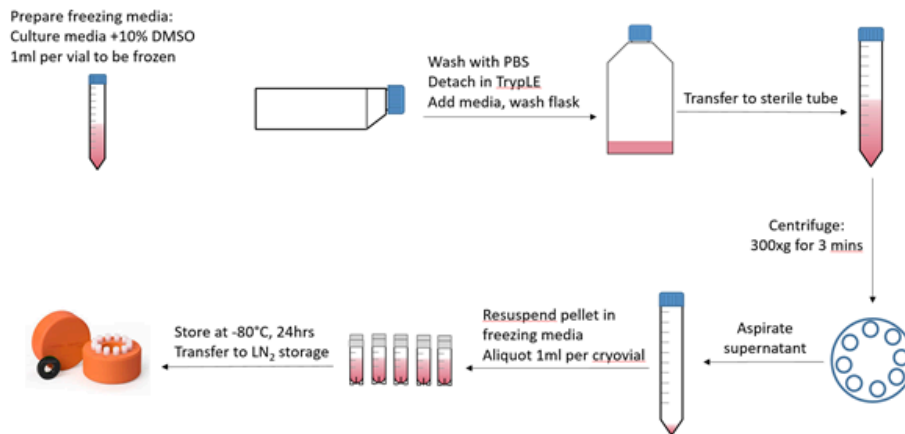
**Protocol Integer ID:** 37464

**Keywords:** freezing cancer cell line, cancer cell lines this protocol, cancer cell line, routine banking of cancer cell line, cancer line, cell, process diagram, ca9

## Abstract

This protocol outlines routine banking of cancer cell lines and Ca9 transduced cancer lines.

Process diagram:



## Guidelines

- As a guideline, we usually bank 5 cryovials from a 70% confluent T150 flask, each containing 1ml cell suspension.



## Materials

### MATERIALS

✕ Falcon™ 15mL Conical Centrifuge Tubes **Fisher Scientific Catalog #14-959-53A**

✕ TrypLE®; Express Enzyme (1X), no phenol red **Thermo Fisher Catalog #12604021**

✕ Nunc®; Biobanking and Cell Culture Cryogenic Tubes, 1.8mL, 48mm, external thread, printed **Thermo Fisher Catalog #375418**

✕ DMSO **Merck MilliporeSigma (Sigma-Aldrich) Catalog #D2650**

✕ DPBS Invitrogen - **Thermo Fisher Catalog #14190**

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

Light Microscope

Microbiological Safety Cabinet (MSC)

Pipette Boy

Stripettes

Pipettes and tips

🔥 37 °C , 5% CO<sub>2</sub> incubator

Centrifuge

CoolCell or appropriate freezing container

-80C freezer


Liquid Nitrogen storage

## Troubleshooting

### Before start



- Pre-warm complete culture media to room-temperature.
- Check the cells under the microscope and record percentage confluency. Cancer cells should be banked when ~70% confluent.



- 1 Prepare  1 mL freezing media per vial as follows: complete culture media + 10% DMSO.
- 2 Detach and collect cells from a flask, by following Steps 1-6 of the protocol: **Passaging adherent cancer cell lines.**
- 3 Aspirate the supernatant, taking care to avoid disturbing the cell pellet. Resuspend the pellet in an appropriate volume of **freezing media**- depending on the number of vials being frozen. Mix well to ensure a single cell suspension.



For example, if 5 vials are being frozen from a T150, resuspend the cell pellet in

 5 mL of freezing media.

- 4 Transfer  1 mL aliquots of the cell suspension to pre-labelled 1.8ml cryovials.
- 5 Place vials in a 'CoolCell' or appropriate freezing container and store at  -80 °C overnight.

#### Note

Appropriate freezing containers will ensure that the liquid freezes at a controlled rate of around

 -1 °C per minute at  -80 °C .

- 6 Transfer the vials to liquid nitrogen for long-term storage.