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Freezing cancer cell lines

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We use this protocol and it's working

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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

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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 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% CO₂ incubator

Centrifuge

CoolCell or appropriate freezing container

-80C freezer

Liquid Nitrogen storage

BEFORE START INSTRUCTIONS

- 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](#)

- 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.