

EXERCISE 3

Cryogenic Storage of Cells

Cryogenic storage is very important for maintaining a continuous cell line. This simple laboratory exercise can be combined with another during the course of the semester.

PROTOCOL

1. Determine the confluency of the plate to be frozen. In order to optimize the recovery of the cells, the plate should be approximately 80% confluent.
2. Remove the media from the plate.
3. Rinse the plate once with serum-free medium.
4. Trypsinize the plate to detach the cells.

Add 1 mL of trypsin to the plate and incubate for 5 minutes at 37°C. Check the plate under the microscope to ensure that a majority of the cells have detached. Pipette the suspension from the plate to a sterile microcentrifuge tube and add 1 mL of 10% serum-media. Centrifuge gently at 5-6 rpm for 3-5 minutes. Carefully remove supernatant.
5. Dispense 0.9 mL of the cell suspension into a sterile cryogenic vial.
6. Add 0.1 mL of DMSO (** BE SURE TO WEAR GLOVES AND NOT TO TOUCH THE DMSO SOLUTION WITH YOUR BARE HANDS! **)
7. Mix gently with a pipette and then cap the vial.
8. Store the vial in -20°C for 4 hours. Then the vial is to be moved to -70°C overnight, and then into liquid nitrogen until use.

“SNAP-THAW” OF FROZEN CELLS FROM LIQUID NITROGEN

1. Warm a vial of serum-containing media in water bath until warm to the touch and add 1 mL of this media to a 35 mm² plate.
2. Remove the cryogenic vial from liquid nitrogen and warm in a 37°C water bath until thawed.

3. Immediately, add the suspension from the cryogenic vial to the plate and incubate at 37°C until attached.
4. Once the cells are attached, gently remove the media and replace with serum-containing media.

MATERIALS

- 10% serum-containing media
Trypsin
Serum-free media
- To make 10% serum-containing media:
 - Thaw the following: 1 L bottle of DMEM-only media (this may or may not contain L-Glutamine), antibiotic/antimycotic solution, and serum (either horse serum or iron supplemented calf).
 - For 1 L: remove 100 mL of DMEM-only.
 - Add 100 mL of serum.
 - Remove 10 mL of solution and use it to dissolve L-Glutamine.
 - Use 2 vials per 1 L bottle.
 - Add back the dissolved L-Glutamine vials into DMEM bottle.
 - Add 10 mL of antibiotic/antimycotic solution.
 - Store at 4°C.
- Trypsin/ EDTA comes as needed for cell detachment; therefore, it only needs to be made into aliquots.

HINTS

- As an additional part of the experiment, cell viability can also be performed using 10X Trypan Blue.

Mix 0.9 mL of the cell suspension with 0.1 mL of 10X-Trypan Blue and count the number of clear cells via a hemacytometer.