Cryopreservation of mammalian cell lines

Cryopreservation is a method whereby cells are frozen, maintaining their viability, until they are defrosted months or years later. Cells are cryopreserved to minimize genetic change and avoid loss through contamination. It is best to cryopreserve cells when they are at their optimal rate of growth.

Materials and reagents

1–2 mL cryovials
CoolCell® freezing unit (BioCision)
Freezing media

Cryopreservation

1. Label cryovials with the date, name of researcher, cell number, passage number and cell type (and any other useful information, for example genetic modifications).
2. If cells are adherent, remove the cell culture media, wash in PBS, add enough trypsin to cover the cells and incubate for approximately 2 min in a 37°C incubator. Resuspend in cell culture media and transfer into a 50 mL Falcon tube.
3. If cells are in suspension, just transfer the desired volume directly into a 50 mL Falcon tube.
4. If required, count cells using a hemocytometer to determine their viability. Cell viability should be at least 75% for cryopreservation.
5. Centrifuge for 5 min at 1,000 rpm at room temperature.
6. Prepare freezing media (Table 1).

   Please note, DMSO is not suitable for all cell types, therefore glycerol can be used as an alternative.

7. Remove the supernatant (keep this; it is needed for the freezing media, see Table 1) and loosen the pellet gently.
8. Add freezing media to the required cell density. For mammalian cells this is usually 1,000,000/mL of freezing media. Cells should not be at room temperature in freezing media for more than 10 min.
9. Aliquot 1 mL into cryovials and secure the lids.
10. Transfer the cryovials into a CoolCell (at room temperature) and put into a -80°C freezer. The CoolCell will ensure that the temperature decreases steadily by 1°C/min.
11. After approximately 24 h. remove the cryovials from the CoolCell and transfer into liquid nitrogen for long term storage.

Culture type	Freezing media	Notes
Cells cultured in FBS-containing media	90% FBS + 10% DMSO	Mix well and warm to 37°C before use.
Cells cultured in serum-free media	90% conditioned media + 10% DMSO	Use the supernatant from the centrifuge step (step 7). Mix well and warm to 37°C before use.
Cells that require glycerol for freezing	90% FBS + 10% glycerol	Mix well and warm to 37°C before use.

Table 1: Different types of freezing media for mammalian cell lines

Thawing frozen cell lines

1. Remove cryovial from liquid nitrogen storage and place in 37°C water bath until only about 80% defrosted (this should take no longer than 1 min).
2. Using a pipette, transfer the contents of the vial into a 15 mL Falcon tube containing about 10 mL of pre-warmed culture media.
3. Centrifuge at 500–1,000 rpm for 5 min, discard supernatant and resuspend in the appropriate amount of cell culture media.
4. Transfer cells into a culture vessel and transfer into a 37°C incubator.