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Cryopreservation of Adherent Cells

The establishment of a cell bank through cryopreservation is essential to maintain cell quality. Continuous cultivation can lead to genetic changes, reduction of proliferation rate, transformation and altered expression patterns. Cryopreservation allows cell lines to be stored almost indefinitely without loss of quality.

The following protocol describes the basic steps for freezing adherent cells. This protocol may not be appropriate for every cell line. Please refer to the protocols of the respective cell banks / sources.

Note:

Follow the national regulations for handling biological materials and wear the appropriate protective clothing.

During the work process, be sure to observe the rules of aseptic technique.

General Information:

Before freezing, the cells should be examined morphologically and checked for contamination. Cells should be in late log phase. For adherent cells, harvesting at 70 – 80% confluence is recommended. A media change should be performed 24 hours prior to freezing.

Freezing Media:

The following media are suitable as freezing media:

Media with Serum:

- 90% culture media +10% DMSO*
- 45% sterile filtered conditioned media / 45% fresh media + 10% DMSO*.

The antifreeze in the media and the high protein content of the serum protect the cells from damage during freezing and thawing. In serum-free media, the protective effect of serum is lost and can be compensated by the addition of antifreeze (e.g., HES, Pluronic F68™, and methylcellulose).

Media Serum-free: (xeno-free, chemically defined)

- 89% chemically defined, serum-free media, 10% DMSO*, 1% Pluronic F68™ [5]
- Commercially available serum-free freezing media

*DMSO is not suitable for certain cells HL-60 [1], HBE4-E5/E7 [1, 2], glycerol may be a suitable alternative

Preparation:

Precool the serological pipettes, freezing media, freezing container, and cryo tubes in the refrigerator.

Procedure:

- Remove all the culture media from the flask and wash the cells twice with warm PBS w/o Ca^{2+} and Mg^{2+} (0.1 mL/cm²).
- Dissolve the cells with a suitable dissociation reagent (e.g., Trypsin, Trypsin-EDTA). Follow the protocol established in your laboratory.
- After addition of fresh media containing a dissociation inhibiting component, transfer the cells into a centrifuge tube.
- Resuspend the cells with fresh medium and determine the total cell count and live cell count (viability).
- Calculate the required volume of freezing medium to achieve a final live cell count of 1 x 10^6 -1 x 10^7 cells/mL
- Centrifuge the cells at 200 x g for 5 min.
- Remove the supernatant and resuspend the cells with the calculated volume of freezing medium.
- Aliquot the cell suspension into the appropriately labelled cryo tubes.
- The cells should be frozen at a controlled freezing rate of -1 °C/minute. You can use programmable freezers or freezing container.
- Store the freezing container overnight in an -80 °C freezer.
- Transfer the cryo tubes the next day to a -152 °C freezer or store them in the gas phase of an LN_2 cryogenic storage unit. Since no reactions take place in the cells from -130 °C [7], storage in the gas phase is possible and recommended for safety reasons [8]
- Check the quality of the cryopreservation 24 hours after freezing by performing the first thawing control (vitality and sterility).

Thawing of the Cells:

- Fill a T-75 cm² flask with 14 mL warm culture medium without antibiotics and 10 mL into a centrifuge tube.
- Remove a cryo tube from the LN_2 cryo storage unit and warm it in a warm water bath equilibrated to the culture temperature of the cell line.
- When ice crystals are no longer visible, transfer the contents of the cryo tube to the centrifuge tube using a sterile pipette.
- Centrifuge the cells at 200 x g for 5 minutes.
- Remove the supernatant and resuspend the cells in 1 mL fresh medium.
- Avoid foaming during resuspension. This can result in poor and uneven cell attachment. Foaming can be reduced by maintaining a steady pipetting rhythm that is not rapid or abrupt.
- Transfer cells to the T-75 cm² flask and incubate for 24 hours.
- After 24 hours, check the cells for vitality, identity, and sterility and sub cultivate if desired.
- The thawing control should be performed periodically.

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