

No. 2: Tips and tricks on freezing cells- Susi and Adi on the freshness associated with cold



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Every culture specialist knows that cells can be frozen and thawed. However, Susi and Adi, as representatives of the suspension and adhesion cells, know of many of their colleagues who have not survived this process. They thus outline below what can happen and how such losses can be prevented.

Most problems tend to occur due to the imbalance with respect to concentration that occurs during the freezing or thawing process. Susi in particular hates being frozen. She well knows the heave-ho types who confuse gentleness with rapidness and tend to forget that rapid freezing leads to the formation of ice crystals in the cell which in turn destroys organelles and the cytoplasm membrane of the cell.

On the other hand, slow freezing often leads to cell death due to dehydration. Thus, slow coaches operating in this way are also dangerous types. If freezing is carried out slowly, local channels and islands are frequently formed in the non-frozen medium so that strong local concentration gradients can result with resulting damage to the cell membrane.

In Adi's opinion, such errors are not at all difficult to avoid. There are a number of additives that can be used to protect cells from such damage. Susi in particular likes additives such as DMSO and glycerol.

Adi likes their cryo-protective properties. They prevent the formation of ice crystals through their high affinity for water. Dextran, polyvinylpyrrolidone and other basic polymers in concentrations of 7 to 12 % volume can be added to stabilise the membrane.

Susi's nucleus-free relatives, the erythrocytes, have a particular liking for dextran in a concentration of 3% volume. Each person working with cultures should find out for himself whether to use DMSO, glycerol or some other additive.

However, Adi maintains that the most important aspect in freezing is proper timing. 1 °C per minute up to - 80 °C is normally optimal. If a special freezing chamber is not available, a suitable vessel can be prepared using a tightly closing polyurethane and a freezer capable of generating - 80 °C.

The following procedure can help save the lives of many of Susi's and Adi's colleagues:

Preparation Prior to Freezing:

Material:

- Cell suspension (either freshly trypsinated cells washed at least twice with fresh complete medium or suspension cultures) should be taken up in medium, if possible at high cell density (between 10E6 and 10E7 cells per ml).
- Closable "cryo tubes" (Bio-freeze vials, centrifuge, PIPETBOY acu, pipettes).
- 2x freezing medium (consisting of complete medium, enriched with at least 10% FCS, whereby DMEM or F-12 is suitable for adherent cells and RPMI-1640 is suitable for suspension cells) with dimethyl sulphoxide as additive. The concentration can be selected between

15 and 20%. This is double the concentration that later reaches the cells.

- 0.5 % volume of a 20% sterile glucose solution may be added to the freezing medium. The solution can also be autoclaved. If absolutely necessary, antibiotics may also be added.
- Warm the freezing medium (2x concentrated) to 37 °C, take up the cells subsequent to trypsinisation (see above) in normal medium and mix in a ratio of 1:1 with freezing medium.
- In our case, 0.9 ml cell suspension in normal medium + 0.9 ml 2x freezing medium.
- Subsequently, briefly centrifuge the freezing tubes containing the cells (2 minutes at 100 g). Aspirate the supernatant leaving approximately 1 ml in the tube.

Now we can proceed to:

Freezing:

Method 1 - Polyurethane box and -80 °C freezer:

Allow the cells to cool in iced water in a refrigerator for approximately 30 minutes. Place the cells in a polyurethane container of wall thickness 2 to 4 cm and close off completely. Place the polyurethane container directly in the freezer (-80 °C). The cells can be stored in this way for several months or, after approximately 24 hours, transferred to a liquid nitrogen container.

Method 2 - Freezing in a nitrogen container using freezing plugs or a biofreezer: Centrifuge the cells briefly (see above) and place them in a freezing tube in iced water in a refrigerator for half an hour. Subsequently, place them in a freezer at -20 °C for 2 hours. Then hang them for 2 to 4 hours or overnight in the freezing plugs in the gas phase of liquid nitrogen. Store the tubes in special racks in the liquid phase of the nitrogen and note the contents and position of the tubes as well as the date of freezing.

Method 3 - Rapid freezing directly in the bottle:

Cells that grow as monolayer cultures in bottles can be frozen in confluent condition directly in the -80 °C freezer. To achieve this, just one change of the normal medium for a single (!) concentrated freezing medium is necessary (just enough to cover the cells). They can then be placed in a polyurethane box and placed in the -80 °C freezer.

Cells frozen in this way can, however, only be stored up to a maximum of 6 months.

Source: Lindl, Toni/Bauer, Jörg: Zell- und Gewebekultur, 2. überarb. und erw. Auflage, Stuttgart/New York, 1989 lines.

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