Cryoconservation of cells

Most cells have a limited life span. After a certain number of cell divisions, the cell division rate decreases and senescence occurs. Primary cultures should therefore not be cultivated for too long. The goal of cryoconservation is the indefinite storage of fresh, biological cell material that is needed at a later time. Storage at below -150 °C allows the preservation of cells for many years. Nevertheless, deep freezing implies stress for the cells, which is why it is urgent to ensure that only vital cells are undergoing this process. To ensure that the cells are viable again after thawing, a number of factors must therefore be taken into account during cryoconservation. To do this, check the following checklist in advance.

- Are the cells in an early passage?
- Do the cells show a typical confluence? Cells in the exponential growth phase are best suited for cryoconservation.
- Do the cells have a vital appearance?
- Can contamination be excluded?

The ROTI®Cell Freezing Medium can be used with any standard cryoconservation protocol. The general freezing method is the same for adherent cells and suspension cells. However, adherent cells must be detached from the culture vessel beforehand. More information can be found in the technical information brochure on cell passage/subcultivation.

Freezing and thawing of cells should always be performed under sterile conditions.

Protocol - Cryoconservation of cells:

1. Preparation:
    a. Determine the desired number of cells to be frozen. This is cell type dependent and should be re-researched beforehand. In most cases, a minimum number of $1x10^6$ to $1x10^7$ cells/ml is recommended.
    b. Label cryotubes (cell type, passage, date) and pre-chill in the refrigerator.
2. Transfer the cells in medium into 15 ml centrifuge tubes.
3. Determine the cell number of viable cells. This should be >90%.
4. Centrifuge the cells for 5 min (approx. 1000 rpm) to obtain a cell pellet.
5. Carefully remove the supernatant.
6. Resuspend the cell pellet with the precooled ROTI®Cell Freezing Medium. Use the appropriate volume to obtain the desired cell concentration. The maximum concentration should be 5x106 to 5x107 cells/ml.
7. Transfer the cell suspension into the prepared cryotubes. **Tip**: For larger volumes, resuspend the solution regularly to keep it homogeneous. Make sure that you fill the cryotubes exactly to the calibration line.
8. The cells should now be frozen slowly and uniformly. The recommended cooling rate is -1 °C/min. This can be achieved either by using programmable freezers or by using freezing aids/isolation boxes (Mr. Frosty), which you place in a -70 °C to -90 °C freezer overnight. **Tip**: Frozen cell material should never be stored between -25 °C and -130 °C for extended periods. This can lead to recrystallization of the ice crystals and thus to the formation of large ice crystals, which will cause significant mechanical damage to the cells.
9. If the cells are frozen, they are stored in liquid nitrogen tanks (< -150 °C) for long-term storage. Wear appropriate protective equipment when transferring.

Protocol - Thawing of cryoconserved cells:

Caution: Antifreeze agents such as DMSO have a toxic effect on cells at room temperature. Accordingly, the individual steps should be carried out as quickly and carefully as possible.

1. Preparation:
   a. Preheat appropriate culture medium and, if necessary, supplements in a water bath at +37 °C.
   b. Prepare culture vessels and centrifuge tubes under the sterile bench.
2. Remove cryoconserved cells from the liquid nitrogen tank with appropriate protective clothing and transfer directly to a vessel filled with dry ice.
3. from the dry ice, warm the cells in the cryotube preferably with a float in a water bath. Make sure that no water gets on the lid of the tube.
4. As soon as the last ice crystals have dissolved, the cell suspension is transferred to a 15 ml centrifuge tube.
5. Immediately afterwards, carefully drop approx. 10 ml of the pre-warmed medium into the centrifuge tube. Very slowly at first, gradually increasing the rate of addition to avoid osmotic shock.
6. Centrifuge the cells for 2-3 min at a lower rotation rate to obtain a cell pellet. The speed depends on the cell type.
7. Carefully remove the supernatant.
8. Resuspend the cell pellet with approximately 10 ml of the pre-warmed medium.
9. Determine the cell number and transfer the cells at the recommended cell density into new culture vessels with pre-warmed culture medium.
10. With the help of a slight north, south, east, west movement of the cell culture flask, evenly distribute the cells and place them in the incubator at +37 °C.

Tip: Alternatively, the cell suspension in step 4 can be seeded directly into a new culture vessel with prepared culture medium (min. 10-20 ml, so that the DMSO is sufficiently diluted). The first change of medium is then carried out after 24 h of cultivation in a +37 °C incubator. Which method is chosen should depend on the sensitivity of the cells.

Note: In this information brochure, the protocols are standard protocols. Please always check for specific requirements of your cells.

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