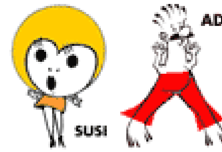
No 3: Tips and tricks on thawing cells and sera - Susi and Adi on the proper way to wake up cells from hibernation



Back to overview

Susi and Adi on the proper way to wake up cells from hibernation

Cell biologists of course also know from their own experience: Those who are rudely awakened tend to be grumpy for the rest of the day. Susi and Adi have experienced similar problems on being thawed subsequent to freezing, although Adi maintains that, in principle, the thawing of cell suspensions is child's play. Susi prefers gentle wakening in a thawing bath. If this process is used however, it should be carried out without unnecessary delay - immediate transfer from freezer to a water bath at 37 ° C. Laboratory personnel should of course be very much awake because, in Adi's opinion, everything must take place quickly. Thus: Keep your eyes open during the thawing process.
As soon as the last ice crystal has melted, the cells should be transferred to a centrifuge tube with 10 times the volume of medium. "This is the time", says Susi, "to freshen up after a long period of being frozen." "All those cryo-protective substances have to be washed out", says Adi. "Therefore, into the centrifuge with them and then on to resuspension in fresh medium."

Thawing cells

Materials:

- Cells in cryotubes in liquid nitrogen
- Nitrogen container
- Protective gloves
- Protective spectacles/face mask
- Water bath (37 °C)
- New culture vessels (COSTAR disposable bottles)
- Complete medium (pre-heated to 37 °C).

Always wear protective gloves and spectacles or face mask.

- Carefully open the N2 container containing the cells, remove the cryotubes (wear protective gloves) from the rack (care: liquid nitrogen may be present in the tubes) and transfer immediately to a 37 °C water bath.
- Retain in the water bath until the last lump of ice has melted.
- Immediately proceed to the laminar flow cabinet, disinfect the outside of the tube with 70 % alcohol and use a 2 ml pipette to transfer the contents to a centrifuge tube containing at least 20 ml of fresh medium.
- Briefly suspend the cells with the pipette, centrifuge at 300 g for 10 minutes and aspirate the medium.
- Fill with fresh medium (10 to 30 ml depending on cell concentration and desired seeding density). Briefly suspend the cells and then transfer to fresh culture bottles.
- Allow to stand for 24 hours without moving.

Thawing and storing sera

This is an important topic for Susi and Adi as serum of course is an important foodstuff for them and their colleagues. If something goes wrong here they have to cope with the consequences. The most important problems are local overheating in the case of too rapid thawing and concentration imbalance which can lead to denaturing of protein in the case of too slow thawing processes.

In Susi's opinion, there is nothing better than healthy high-quality food. In Adi's opinion, this is frequently not the case, mainly due to errors in storing sera and not so much due to the selection and composition of the sera involved. "As a rule of thumb", Adi says, "the lower the temperature the longer the storability and hence the lower any loss in quality during storage." At 4 °C sera can be stored over a period of 1 to 2 weeks without losing much activity. At - 20 °C, sera should not be stored for longer than 1 year. At - 40 °C storage time can be extended to 2 years and at - 60 or - 80 °C, three years' storage is possible.

A further criterion for storability is the content of haemoglobin. Haemoglobin is the most important auto-oxidation factor and, even when frozen, it binds oxygen molecules and can thus cause considerable damage to the serum. In Adi's opinion, repeated freezing and thawing processes should be avoided as these inevitably lead to loss in quality of the serum.

In this connection, glass bottles should be preferred to plastic containers as they are less gas- permeable.

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

Back to overview