

## **Cryopreservation of cells**

Hybridomas , as many other cultured cells, can be kept frozen in liquid nitrogen for many years.

Because hybridomas are vulnerable in the first weeks after their establishment and can easily be lost, it is a safe practice to freeze a few vials of cells right after the identification of the desired hybridomas. After the isolation of an active clone, grow a large batch of cells and freeze aliquots in a few dozen vials.

These will serve as a safety stock.

## **Freezing of cells**

Freezing medium: DMEM + 25%-50% serum + 10% Dimethyl sulfoxide (DMSO) sterilized by filtration. Keep cold at 4°C

1. Harvest cells and remove supernatant.
2. Resuspend cells in freezing medium to approximately  $10^7$  /ml. Aliquot 1 ml per sterile 2 ml tube with screw cap ( 38x12.5). close cap tightly.
3. Freeze cells slowly, either in a special apparatus designed for controlled freezing which fits the union carbide liquid N<sub>2</sub> containers, or wrap the vials in gauze and place in a polystyrene foam box which will then be left for 12 hours in a deep-freeze (-80°C). Tubes should then be transferred into liquid nitrogen where they can be kept for a long time.

## **Thawing cells**

1. Thaw frozen cells quickly at 37°C in a water bath.
2. Clean the tube with 70% alcohol.
3. Immediately when thawed, transfer the cells on top of 10 ml cold growth medium.
4. Centrifuge cells, resuspend pellet in growth medium ( 10 ml ), and culture in two flasks.