

CELL FREEZING INFORMATION

In order to allow Glycotope Biotechnology to establish your cell line in culture quickly, it is essential that the cell line is frozen under optimal conditions. The import of bovine material or blood or serum components from animal or human origin is restricted by European and German authorities. To circumvent any problems for the shipment at customs we recommend to replace fetal calf serum (FCS) by bovine serum albumin (BSA) during the freezing procedure. In our hands the following protocol worked well for a broad range of mammalian cells.

1. Material

Cryotubes, 2 ml (e.g., Greiner # 122277)

Dimethyl sulfoxide (DMSO) (cell culture quality; e.g., Hybri-Max, Sigma D-2650)

Bovine serum albumine (BSA) (e.g., AlbumMAX II, Life Technologies # 11021-029)

2. Cryopreservation

1. Prepare an ice water bath.
2. Prepare a expendable polystyrene (Styropor) box filled with dry ice.
3. Prepare a sufficient amount (100 µl/tube) of DMSO/medium (1:1) and cool it on ice.
4. Prepare freezing medium (+ 2 % BSA; 1 ml/tube) and cool it on ice.
5. Collect cells from a logarithmic growing culture (80 to 90 % of full density) by centrifugation (RT, 600 min⁻¹, 4 minutes)
6. Resuspend the cells in cold freezing medium (+ 2 % BSA) in a final concentration of 2 to 5 x 10⁷ cells/ml and place for 10 minutes on ice.
Not more than 10 cryotubes should be processed parallel.
7. Transfer 1 ml of cell suspension to a 2 ml cryotube.
8. Add 100 µl of DMSO/medium mixture, close the tube, mix immediately and transfer to the dry ice box. This step has to be performed within 90 seconds.
9. Transfer the cryopreserved cells to a -70 °C freezer.
10. The cells can be stored for up to 2 weeks at -70 °C and shipped on dry ice. For long term storage the cells a transferred to liquid nitrogen cell storage systems.