

Freezing and thawing cells

Freezing medium:

- DMSO 10% (sterile, cell culture grade)
- FCS 20%-90% depending on the cell type (typically 20%)
- Adjust with cell culture medium.

Trypsinize a T75cm² flask or a 60cm² dish as usual.

Resuspend cells in 5 ml medium

Spin down cells 5 min at 800 rpm

Gently resuspend cell pellet in 1 ml cold freezing medium and transfer to cryotube

(NUNC 1.8ml cat # 368632)

Store at -70C for ~2days

Transfer to liquid N2 tank.

To thaw cells, quickly thaw a cryotube at 37C, add 10 ml of warm medium and spin down. Discard sup, resuspend cells in 10-20 ml medium and plate.

For 10 ml:

- 8.75 ml 8M Guanidine HCl
- 0.2 ml 1M Tris pH 7.5
- 0.1 ml 1M DTT
- 0.95 ml H2O

- Incubate membrane at RT for 20 minutes in Stripping buffer
- wash with water, until the membrane does not smell DTT
- block membrane and reprobe.