Freezing CHO Cells

Christina McGee 4-5-2012

0) Grow 10 cm dish to 50-90% confluency in T-75 flask

1) Digest cells with 3 ml Trypsin/EDTA, wait for cells to detach.

2) Add 7 ml media, transfer to 15 ml sterile tube.

3) Centrifuge 250 G/5 min.

4) Remove supernatant. Gently resuspend cell pellet in 2 ml freezing medium*.

5) Transfer 0.5 ml to 4 freezing vials labeled with name of cell line, date and your initials.

6) Place vial in freezing box in -80°C freezer.

7) Transfer to liquid nitrogen 1-4 days later.

8) Record location of cells in Liquid N2 excel spreadsheet

*Freezing medium: Cell medium (F-12 or DMEM/F-12) without selection agents or antibiotics plus 20% FBS and 10% DMSO (dimetylsulfoxide, sterile).