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Standard Operating Procedures: Cell Freezing

Office of Environmental Health and Safety, Northeastern University

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CELL FREEZING

References:

1. *Freshney, Culture of Animal Cells handbook, 4th edition, page 158*

MATERIALS AND REAGENTS

1. Cells in log phase of growth (~ 2 weeks after passage)
2. PBS (Ca and Mg free) and 0.25% trypsin
3. Growth medium with 50% FBS
4. DMSO
5. 5-ml syringe
6. Cryovials
7. IPA
8. Hemocytometer
9. Insulated freezing container

PROCEDURE

1. Remove culture media from the cultures, discard.
2. Trypsinize (5 min with 0.25% trypsin at 250 µl of trypsin solution per 1 ml of culture medium removed)
3. Count and centrifuge.
4. Resuspend the cells in freezing medium (10% DMSO and 90% of growth medium with 50% FBS) at 1×10^6-1×10^7 cells/ml. A delay of up to 30 min at room temperature is not harmful when using DMS).
5. Dispense the cell suspensions into prelabeled cryovials.
6. Feel the freezing container with IPA as indicated in the instructions printed on the outside of the container. Place the ampules in the container.
7. Place the container in the -80degC freezer overnight.
8. Transfer the cryovials to a liquid nitrogen freezer. The transfer must be done quickly because the cells deteriorate if the temperature raises above -50degC.