Paul K. Wallace, Ph.D.
Department of Flow and Image Cytometry
Roswell Park Cancer Institute

FREEZING MONONUCLEAR CELLS

PRINCIPLE

Cryopreservation of mononuclear cells inhibits metabolism while maintaining viability. Dimethyl sulfoxide (DMSO, a cryoprotective medium) is used to prevent ice crystal formation and maintain viability. Cells are frozen using a control rate freezer, removed to a -80°C freezer and, after 3 or 4 days, moved to long term storage in liquid nitrogen at or below -120°C.

MATERIALS AND REAGENTS

Fetal Bovine Serum	14-501F	BioWhittaker
Dimethyl sulfoxide (DMSO) (Cryoserv)		Research Industries Corp.

PROCEDURE

1. Ficoll peripheral blood to obtain mononuclear cell suspension following standard procedure.

2. Wash once in HBSS centrifuging at 1500 rpm for 10 min.

3. Between the first and second wash determine the number of cells on the ACT10

4. Wash once in HBSS centrifuging at 1400 rpm for 5 min.

5. Resuspend the cells at 1 - 2 x 10^7 cells per ml in cold fetal bovine serum. Keep cells on ice.

6. Prepare the freezing media, a 20% solution of DMSO in fetal bovine serum. The volume will depend on the volume of cells to freeze.

7.. Slowly (at a rate of 1 drop every 5 seconds), with gentle mixing and keeping the cells on ice, add a volume of DMSO/fetal bovine serum equal to the volume of fetal bovine serum in step 5.

8. Place the cells in a alcohol bath "Mr. Frosty" previously equilibrated to 4°C and place the cells and Mr. Frosty in a -70oC freezer for 2 -3 days.

9. After 2 - 3 days, place cells in the -150°C freezer or liquid Nitrogen carboy.

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