PURPOSE
Ficollated peripheral blood mononuclear cells (PBMC) are cryopreserved for future analysis.

EQUIPMENT/SUPPLIES
1. Sterile 50 ml polypropylene tubes
2. 37°C water bath
3. 10 and 25 ml pipets, individually wrapped
4. 2 ml cryogenic vials
5. -80°C freezer
6. Freezing containers (Nalgene)
7. Liquid Nitrogen freezer
8. Hemacytometer

REAGENTS
1. Human AB Serum
2. DMSO (Cat# D2650, endotoxin tested, Sigma) DMSO expires 6 months after opening.
3. RPMI 1640 (Life Technologies)
4. Human Serum Albumin (5 grams, cat# A5843, low endotoxin, Sigma, or cat# 800-125P Gemini Bio-Products)
   To make 12.5% HSA solution, add 40 mls RPMI 1640 in the 5 gram bottle. Mix gently by inversion. Do not vortex as HSA will foam up. Filter sterilize and store at 4°C for up to 6 months.
5. Assay Media: MATIS + 10% Human AB serum
   Pipet into a sterile 500 ml bottle:
   250 mls RPMI 1640
   250 mls EHAA
   10 ml Pen/Strep solution
   25 mls of 200 mM L-Glutamine
   50 ul of 0.5 M 2-Mercaptoethanol solution
   50 mls heat inactivated human AB serum
   Label the bottle with date and initials. Store at 4°C.
6. Trypan Blue (0.4% in PBS, cat# T8154 Sigma)
PROCEDURE:

**Freezing PBMC**

1. Place Nalgene freezing containers at 4°C.

2. Label cryovials with:
   - Patient ID
   - 10 x 10^6 PBMC
   - Blood draw date and Tech initials

3. Place labeled cryovials in -20°C freezer.

4. For each cryovial, 1 ml of total volume is added per 10 x 10^6 PBMC.

5. Prepare 2X freezing media: for 20 cryovials
   - 10 mls 12.5% HSA solution
   - 2.5 mls DMSO
   Mix and place on ice for a minimum of 30 minutes.

6. Resuspend ficoll PBMC at 2 x 10^7 viable lymphocytes/ml in cooled 12.5% HSA solution.

7. Add the chilled 2X freezing media to the cell suspension dropwise, while gently by swirling the tube.

8. Remove chilled cryovial from -20°C and aliquot 1 ml per cryovial.

9. Place cryovial on ice once PBMC is aliquoted.

10. Transfer cryovials into Nalgene freezing container and place in a -80°C freezer.

11. For long-term storage, transfer the cryovials into liquid nitrogen freezer after 24 hours of freezing at -80°C. Never store cells at -20°C, even temporarily.

12. Record the vial location (box number and slot number) on both the specimen database and freezer binder.
Thawing PBMC

1. Place the cell culture media at 37°C water bath for about 30 minutes.

2. Label a 50 ml centrifuge tube per sample and add 8 mls of warm (22°C to 37°C) assay media.

3. Remove cryovials from liquid nitrogen freezer and place directly on dry ice.

4. Thaw no more than 2 cryovials at a time. Place cryovials in a 37°C water bath until cell suspension is almost completely melted or a small bit of ice remains.

5. Dry off the outside of the cryovials and wipe with 70% ethanol.

6. Add 1 ml of warm (22°C to 37°C) cell culture media to the thawed cells slowly.

7. Transfer the cell suspension to the 50 ml polypropylene tubes containing 8 mls of media.

8. Balance tubes and centrifuge at 1200 rpm for 10 minutes.


Approvals

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