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SINGLE FICOLL PROCEDURE TO ISOLATE MONONUCLEAR CELLS

PRINCIPLE

This procedure describes a method for isolation of mononuclear cells from circulating blood. Histopaque®-1077 is a solution of polysucrose and sodium diatrizoate, adjusted to a density of 1.077±0.001 g/mL. This medium facilitates rapid recovery of viable mononuclear cells from small volumes of blood.

Anticoagulated blood is layered onto Histopaque®-1077. During centrifugation, erythrocytes and granulocytes are aggregated by polysucrose and rapidly sediment; whereas, lymphocytes and other mononuclear cells remain at the plasma-Histopaque®-1077 interface. Erythrocyte contamination is negligible. Most extraneous platelets are removed by low speed centrifugation during the washing steps.

REAGENTS AND SUPPLIES

Histopaque®-1077
RPMI 1640
RPMI 1640 w/ 10% FCS
Fetal calf serum (FCS) or autologous serum
15 mL polypropylene tubes

PROCEDURE

Ficoll 1077 Separation

1. 1. Dil. Blood with HBSS, PBS or RPMI w/o FCS.
   - (1:1 dilution, 1 part blood to 1 part media)

Calculate the amount of Ficoll 1077 needed using a diluted sample to Ficoll ration of 2:1.

Ex.
With 8 mL of diluted sample use 4 mL Ficoll.

2. 2. Put Ficoll in bottom of tube
   - 15 ml polypropylene 4 mls of Ficoll, 8 ml of diluted blood
   - 50 ml polypropylene 15 ml of Ficoll, 30 ml of diluted blood

3. Carefully layer sample over each tube of Ficoll. Do not pipette the sample too quickly as mixing will occur. Wash the sample tube with a small amount of RPMI 1640 media and add to the Ficoll tube.

4. Centrifuge the samples for 30 minutes at 1,200 rpm and 25°C without the brake.

5. After the centrifuge has stopped, promptly remove cells from the Ficoll and proceed with washing because the Ficoll solution is toxic to cells.

   a. The mononuclear layer should be visible as a cloudy white disk at the interface of the plasma-Ficoll layers, roughly in the middle of the tube. Carefully

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remove and discard the top layer of plasma and media until there is only a small amount left above the cell layer.

b. Remove the mononuclear layer (the cloudy disk) and place in a sterile tube. Be careful not to disturb the red cell layer at the bottom of the tube.

6. Dilute the mononuclear cells with 3 to 6 times their volume using RPMI 1640 w/10% FCS.

7. Centrifuge for 10 minutes at 1,500 rpm and 25°C with the brake.

8. If the supernatant media is very cloudy after centrifugation, transfer the supernatant to another sterile tube, dilute further with RPMI 1640 w/10% FCS and recentrifuge.at 1,200 rpm and 25°C with the brake.

9. Aspirate the supernatant and flick the tubes to resuspend the pellets.

10. Resuspend the cells with RPMI 1640 w/10% FCS to 2 to 5 mL, depending on the size of the pellet, and recentrifuge at 1,200 rpm for 5 min at 25°C with the brake.

11. During recentrifugation perform a cell count and viability.

12. Proceed with experiment or lyse to remove any red cells present.

Lysing Red Blood Cells

1. After resuspending the cell pellet, add 1 to 3 mL of sterile water and gently shake.

2. Allow to stand for 15 to 30 seconds.

3. Immediately add an equal volume of 2X PBS and shake, bringing the solution back to a buffer.

4. Dilute with RPMI 1640 w/10% FCS.

5. Centrifuge for 10 minutes at 1,200 rpm at 25°C with the brake.

6. Remove the supernate and resuspend in RPMI 1640 w/10% FCS.