

SOP-P035

Dextran Sedimentation

Objective: To isolate neutrophils, from whole blood for cell function tests. If pure neutrophils are required it is necessary to ficoll the blood first and perform the dextran on the red blood cell portion at the bottom of the tube.

Procedure:

1. Ficoll the blood following SOP#-P032 for ficoll hypaque procedure, through step #8.
2. Using a pastuer pipet remove all layers to just above the red cell layer and discard. Do not aspirate too close to the red blood cell layer since the granulocytes are laying right on top of them.
3. Vortex, or use a pipet to mix up the red blood cells (RBC's)and granulocytes which are now what is left in the 15cc tube.
4. Using a serological pipet add 10ml Phosphate Buffered Saline (PBS) to the RBC's/grans. See SOP#-P020 for preparation of PBS.
5. Using a serological pipet add 3ml 3% Dextran T-500. See SOP#-P036 for preparation of 3% Dextran..
6. Mix gently by inversion.
7. Place in 37° water bath for 45minutes. Gently tap the top of the tube a couple of times during this incubation.
8. Using a pasteur pipet aspirate the supernatant and transfer to a new 15cc centrifuge tube.
9. Centrifuge at 200xg for 10 minutes.
10. Decant and resuspend the button.
11. Lyse the remaining RBC's by adding 2ml of millipore filtered water, gently shake for 20 seconds and IMMEDIATELY add excess (minimum 12ml) PBS or HBSS(Ca²⁺ and Mg²⁺ free). See SOP#-P011 for calcium and magnesium free HBSS preparation.
12. Centrifuge for 10 minutes at 200xg.
13. Wash twice in HBSS (Ca²⁺ and Mg²⁺ free) containing 15% FCS (Fetal Calf Serum). Alternatively use PBS.
14. Resuspend the cells in a known volume of PBS.
15. Do a cell count on the Coulter Counter. See SOP#P005 for operation of the Coulter Counter.
16. Adjust to desired cell concentration.

NOTE: Preparation should be 96-98% pure neutrophils.
