

Basic SOP for purifying PBMCs from Whole Blood (to be modified with specifics for each individual study)

PROCESSING INSTRUCTIONS:

For Whole Blood collected in CPT tubes;

- Tubes should be kept at room temperature and spin started within 2 hours of collection, if possible.
- Estimate the volume of blood received by comparison to marked sample tube. Record on appropriate lab collection form.
- Gently invert the tubes a few times to mix the blood. Centrifuge at 1700 x g for 24 minutes at room temperature (23°C). Okay to have brake on.
- After centrifugation, there will be several distinct layers in the tube, separated by the inert gel. Below the gel will be the RBCs and granulocytes. Above the gel will be the diluted plasma and a layer of white cells (mainly PBMCs). If layers are not present, 'poke' the gel with a 1 ml pipet, then spin tube again.
- Suction off most of the upper layer. Using a sterile transfer pipet, transfer the PBMC layer to a sterile 50 ml centrifuge tube containing ~1X PBS. Collect cells from additional tubes of same subject and add to 50 ml conical. Bring volume to 45-50 ml (need 15 ml wash volume per original CPT tube, so cells from 3 CPT tubes may be combined into one wash tube).
- Centrifuge at 300 x g for 15 minutes at room temperature.
- The PBMCs will pellet at the bottom of the tube. Suction off the supernatant.
- Add 1 ml washing medium per tube and gently resuspend the cells. If there are multiple wash tubes, combine all cells into one tube and remove an aliquot for counting. If necessary, redistribute cells to multiple tubes for the second wash. Each tube should have 10 ml of washing medium per original CPT tube or a maximum of 5 x 1 ml of combined cells. Bring volume in wash tubes up to 30-50 ml (dependent on how many tubes were combined). Mix thoroughly. Cap the tube(s), and gently invert to mix.
- Repeat the centrifugation step for 10 minutes. While the cells are spinning, count from the reserved aliquot. Calculate volume of freezing medium needed for desired concentration(s). When spin is finished, carefully remove the supernatant without disturbing the pellet.
- If count was not already done, or needs repeating, combine all tubes into one. Count. Top tube up with 1XPBS and pellet for 10 minutes at 300 x g.
- Resuspend the pellet in desired volume of freezing medium. Resuspend to ~ 10^7 per ml, or appropriate concentration for the requirements of the study. Aliquot to cryovials. Each cryovial should contain 1 ml of cells in freezing media.
- Slow freeze in a freezing container (such as a Mr. Frosty) at -80°C. Note the # of vials and the # of cells per vial in the log.
- After at least 4 hours, or up to 72 hours, transfer the cryovials to the -150°C freezer or to liquid nitrogen.

Isolation of PBMCs from Whole Blood using Barrier Tubes

- Prepare SepMate-50* tubes by pipetting 15 ml Ficoll-Paque Plus or Lymphoprep Density Gradient medium into the tube. If medium is on the walls of the tube, centrifuge briefly (1000 x g, 1 minute, room temp). The top of the medium will be above the insert in the SepMate tube. (Tubes may be prepared ahead of time; if stored at 4 degrees, allow tubes to warm to room temperature before use).
- Prepare the sample by diluting whole blood with an equal volume of 1X PBS.
- Add 30 ml diluted blood to each SepMate tube (allowable volumes: 5-17 ml of initial sample; 10-34 ml diluted sample. Minimum RBC volume is 2 ml and maximum RBC volume is 12 ml; subjects known to have a high or low hematocrit may require more or less blood per tube). Pour or pipet slowly down the side of the tube; some mixing with the medium above the insert will occur.
- Spin at 1200 x g, 10-20 minutes, room temperature, brake ON. If RBCs are present above the barrier after the spin, an additional 10 minute spin may be done; this shouldn't be necessary with an initial 20 minute spin.
- Pour off the upper layer into a new 50 ml tube. Be careful not to keep the tube inverted for more than 2 seconds. Alternatively, remove most of the plasma layer and then collect the cell layer into a new tube.
- From this point, two washes should be done, following the same guidelines as for cells from CPT tubes. One SepMate tube, with 30 ml diluted blood is the equivalent of about 2 CPT tubes. First wash should be at least 30 ml; second wash should be at least 20 ml. Tubes may be combined for counting and washing, as needed.

Note: Greiner Leucosep tubes have a different recommended protocol; this procedure is specifically for StemCell Technologies SepMate-50 tubes. The gradient medium will be just below the barrier in a Leucosep tube. The Leucosep tubes may be used without dilution of the blood, but a 1:2 dilution is recommended if desired. Leucosep tubes recommend a first spin with no brake, and have slightly different spin times and g forces.