

Lymphocyte Collection

Whole blood sample should be collected in a yellow top Vacutainer™ tube containing ACD (acid-citrate-dextrose) additive solution, which compliments inactivation.

Step 1 – Pour whole blood sample into red conical Falcon™ tube (or other collection tube that can be used in a standard clinical centrifuge).

Step 2 – Add Purgene™ RBC Lysis Solution to the whole blood specimen into the Falcon™ tube at a ratio of 3:1 (e.g. 22.5 ml Purgene™ RBC Lysis Solution to 7.5 ml whole blood).

Step 3 – Tilt gently several times to mix whole blood with Purgene™ RBC Lysis Solution and let sit for 5 minutes at room temperature. Continue to tilt gently after the 5 minutes.

Step 4 – Centrifuge for 5 minutes at 2000 rpm. Remove supernatant leaving behind the visible white cell pellet and about 150-300 µl of the residual liquid.

Step 5 – Vortex the tube vigorously to resuspend the cells in the residual liquid. Add approximately 2 ml of PBS solution and continue to vortex the tube.

Step 6 – Centrifuge for 2 minutes at 2000 rpm. Remove supernatant leaving behind the visible white cell pellet and about 150-300 µl of the residual liquid.

Step 7 – Break up the white cell pellet and add to a plastic cryogenic vial. Add one pipette full of PBS solution to Falcon™ tube to wash the remnants and add to the cryogenic vial.

Step 8 – Label cryogenic vial with the patient's identification number. Vials of lymphocyte should be frozen and maintained by placing them in a -80 °C freezer. **Do not submerge vials in liquid nitrogen.**