Genorise Red Blood Cell Lysis Buffer

Genorise Red Blood Cell Lysis Buffer is to isolate white blood cells from 0.3 ml of blood for isolations of DNA or protein, and is for 1000 applications. If a smaller or larger sample volume is employed, the reagent quantity should be proportionally decreased or increased. This reagent can also be used to clear up red blood cells following isolation of white Buffy coat. The white cell isolate can also be used for cell culture purpose without affecting white cell viability. This kit will guarantee the quality and quantity of white blood cells and is much more cost effective than the similar products.

Materials provided in the kit:
1000 ml Red Blood Cell (RBC) Lysis Solution

Materials required but not provided:
PBS, pH 7.4, prepared in sterile distilled deionized water.

Protocol

Isolation of White Blood Cells

1. Take 0.3 ml whole blood to a 1.7 ml microcentrifute tube, and add 0.9 ml of Red Blood Cell Lysis Buffer, vortex for 20 sec.
2. Incubate at room temperature for 10 min to completely disrupt the red blood cells.
3. Centrifuge the tube at 3000 x g for 3 min at 4ºC and discard the supernatant.
4. Add 1 ml of cold PBS to the white pellet, suspend the cells by a pipette, and centrifuge the tube at 3000 x g for 3 min at 4ºC.
5. If the white pellet contains red substance, add 0.3 ml RBC Lysis Solution, suspend the cells by a pipette, and incubate at room temperature for 5 min.
6. Centrifuge the tube at 3000 x g for 3 min at 4ºC and discard the supernatant.
7. Repeat Step 5 and 6 until the pellet is red-free.

Alternative protocol: Removal of red blood cells from white Buffy coat

1. Collect 10ml blood in a 10ml glass tube containing anti-coagulant such as heparin, centrifuge 10 min at 2000g.
2. Pick up the white Buffy coat layer by a Pasteur pipet and place into a 15ml conical tube containing 4ml red blood cell lysis buffer, briefly vortex to suspend the cells.
3. Incubate 10 min at room temperature.
4. Spin 5min at 2000g to pellet the white blood cells and discard the supernatant.
5. Remove the liquid residue and add 1 ml red blood cell lysis solution to suspend the white cells.
6. Transfer the cells to a 1.5 microcentrifuge tube and spin 1 min at 3000g.
7. Repeat step 5 and 6 until red color disappears (isolated cells are free of red cells).
8. Completely remove the supernatant and suspend the cells with 1ml PBS.
9. Spin 1 min at 3000g and completely remove the PBS.