Genorise Red Blood Cell Lysis Solution (10x)

Genorise Red Blood Cell Lysis Solution (10x) is to isolate white blood cells from 0.3 ml of blood for isolations of DNA or protein, and is for 10000 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 reagent will guarantee the quality and quantity of white blood cells and is much cheaper than the similar products.

Materials provided in the kit:
1000 ml Red Blood Cell (RBC) Lysis Solution (10x)

Materials required but not provided:
PBS, pH 7.4

Protocol

Isolation of White Blood Cells

1. Dilute the Genorise Red Blood Cell Lysis Solution (10x) for 10 times. For example, mix 100 ml of the Genorise RBC Lysis Solution (10x) with 900 ml of sterile distilled deionized water and use the diluted solution for removal of RBC in the blood.
2. Take 0.3 ml whole blood to a 1.7 ml microcentrifute tube, and add 0.9 ml of Red Blood Cell Lysis Solution, vortex for 20 sec.
3. Incubate at room temperature for 10 min to completely disrupt the red blood cells.
4. Centrifuge the tube at 5000 x g for 5 min at 4ºC and discard the supernatant.
5. Add 1 ml of cold PBS to the white pellet, suspend the cells by a pipette, and centrifuge the tube at 5000 x g for 5 min at 4ºC.
6. 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.
7. Centrifuge the tube at 5000 x g for 5 min at 4ºC and discard the supernatant.
8. Repeat Step 6 and 7 until the pellet is red-free.

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

1. Collect 10 ml blood in a 10ml glass tube containing anti-coagulant such as heparin, centrifuge 10 min at 2000 x g.
2. Pick up the white Buffy coat layer by a Pasteur pipet and place into a 15ml conal tube containing 4ml red blood cell lysis solution, briefly vortex to suspend the cells.
3. Incubate 10 min at room temperature.
4. Spin 5 min at 2000 x g 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 3000 x g.
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 ice cold PBS.
9. Spin 1 min at 3000 x g and completely remove the PBS.