DIRECT STAINING PROTOCOL

Whole blood method

If whole blood is being used, erythrocytes have to be lysed to not interfere with FACS analysis.

The Diaclone lysing solution (Cat N° 858 040 125) is an erythrocyte lysing solution used for the preparation of leukocytes in whole blood for their analysis by flow cytometry. This procedure allows defining populations of lymphocytes, monocytes and granulocytes by forward and side scatters without isolating the cells populations before analysis. Diaclone lysing solution can be used for either "no-wash" or "wash" staining procedures.

Each vial contains 3g of lyophilised reagent for 125 tests. Store at 2 to 8°C.

Reagent preparation

1. Reconstitute with 250 ml of distilled water.
2. Check and adjust the pH to 7.3.
3. After reconstitution, the lysing solution is stable for 12 months

Staining and lysing protocol

1. Add 50 µl of EDTA treated blood to a reagent tube
2. Add 10 µl of conjugated mAb
3. Vortex the tube and incubate for 15 min at room temperature in the dark
4. Add 2 ml of lysing solution to each tube
5. Mix by vortexing and incubate for 10 minutes at room temperature in the dark
6. Centrifuge the cell suspension for 2 minutes at 828g
7. After centrifugation, remove supernatant and dispense 200 µl of PBS
8. Analyse by flow cytometry within three hours
9. When the samples are not immediately analysed, keep the tubes at 4°C in the dark

Isolated leukocytes

PBMC are isolated from whole blood by Ficoll-Hystopaque density gradient separation as described below.

Ficoll performance

1. Safety precautions should include gloves when working with blood
2. Add 15 ml of blood to 15 ml of 0.9% NaCl in a 50 ml centrifuge tube
3. Layer 15ml of Hystopaque -1077 (SIGMA H-8889) under 30 ml of cell suspension
4. Centrifuge at room temperature at 1193g for 20 min or at 828g for 30 min.

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Isolation of PBMC

1. Mononuclear cells form a visible clean interface between plasma and Hystopaque
2. Aspirate the mononuclear cell layer. Collect cells in a clean 50ml centrifuge tube
3. Fill the tube with 0.9% NaCl, mix the cells, and then spin at 828g for 5 min.
4. Aspirate and discard supernatant
5. Resuspend the cells in 0.9% NaCl, mix and spin at 1864g for 8 seconds
6. Aspirate and discard supernatant
7. Fill the tube with 0.9% NaCl, mix the cells, then spin again at 828g for 5 min.
8. Suspend cells with 10 ml of RPMI 1640 10% FCS, mix gently and determine cell viability
9. Cells can be used for biological assays, for immunofluorescence tests

Staining assay

1. Add 50 µl of the cell suspension (1.10^6 cells) to each microtiter plate well
2. Add 10 µl of conjugated mAb
3. Incubate for 30 min at 4°C in the dark
4. Wash twice with PBS containing 1% BSA and 0.1% NaN_3
5. Resuspend cells in 50 µl PBS
6. Add 200 µl of PBS 1% paraformaldehyde
7. Analyse by flow cytometry or store the cells at 4°C

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