immunostep
we know how

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Direct Immunofluorescence of Whole Blood Using a Lyse/Wash Before Staining Protocol

1. Transfer 100 µL of anticoagulated (EDTA) blood to a 12 x 75 mm test tube (10^6 cells).
2. Add Red blood cells lysing solution, according to the manufacturer's instructions to each sample and mix gently with a vortex mixer.
3. Centrifuge at 540xg for 5 minutes and carefully aspirate the supernatant so as not to touch the cell pellet. Leave 50 µl of non-aspirated liquid.
4. Add 2 mL of wash solution and resuspend the cells. Mix well.
5. Centrifuge at 540xg for 5 minutes and carefully aspirate the supernatant so as not to touch the cell pellet. Leave 50 µl of non-aspirated liquid.
6. Add the appropriate volume of the antibody and mix gently with a vortex mixer. The optimal volume should be determined by the individual laboratory. The recommended negative control is an appropriate isotype control.
7. Incubate in the dark at room temperature (20-25 °C) for 15 minutes for 30 minutes at 4°C.
8. Add 2 mL of wash solution and resuspend the cells. Mix well.
9. Centrifuge at 540xg for 5 minutes and carefully aspirate the supernatant so as not to touch the cell pellet.
10. Resuspend the pellet in 0.3 ml of flow cytometry solution.

Acquire on a flow cytometer or store in the dark at 2 - 8°C until the analysis is carried out.

If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the product is suspected, contact our Technical Services. (tech@immunostep.com)

Reagent list:

- Wash solution: 20 Mm NaH_2PO_4, 150 NaCl, pH 7.2 + 0,09% Sodium azide (NaN_3) + 0,5 % bovine serum albumin.
- Red blood cells lysing solution (if necessary)
- Flow cytometry solution: 20 Mm NaH_2PO_4 , 150 NaCl, pH 7.2 + 1% Paraformaldehide.
- Isotype control: http://immunostep.com/22-isotype-controls