we know how immunostep

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Indirect Immunofluoresce Cell Surface Staining Protocol

1. Add the suggested volume indicated on the antibody vial to a 12x75mm cytometer tube. It is advisable to prepare an additional tube with the appropriate pure isotype control.
2. Add 100 µL of sample (up to 10^6 cells) and mix gently with a vortex mixer.
3. Incubate in the dark for 15 minutes at room temperature (20-25°C) or for 30 minutes at 4°C.
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 secondary conjugated antibody and mix gently with a vortex mixer. Incubate in the dark at room temperature (20-25 °C) for 15 minutes or at 4 °C for 30 minutes.
7. If necessary, (i.e bone marrow, venous blood samples...) add Red blood cells lysing solution according to the manufacturer's instructions to each sample and mix gently with a vortex mixer.
8. 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.
9. Add 2 mL of wash solution and resuspend the cells. Mix well.
10. Centrifuge at 540xg for 5 minutes and carefully aspirate the supernatant so as not to touch the cell pellet.
11. 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