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Platelets Immunofluorescence Staining Protocol

A. Procedure for preparation of ACTIVATED PLATELETS.

1. Centrifuge a tube of freshly drawn (Citrate) blood at 75xg for 20 minutes.
2. Remove platelets (top layer), and wash twice by adding 2 mL of wash solution and resuspend the cells. Mix well.
3. Add thrombin to cell suspension (1-0,2 U/ml) or Phorbol 12-myristate 13-acetate (PMA) (25µg/ml).
4. Incubate in the dark for 10 minutes at 37°C. After that, incubate the sample at 4°C for 10 minutes.
5. Transfer 5 µL of activated platelets to a 12 x 75 mm cytometer tube. A recommended control is a tube with non-activated platelets.
6. Add 95 µL of wash solution and mix well.
7. Add the appropriate volume of the antibody and mix gently with a vortex mixer. The optimal volume should be determined by the individual laboratory.
8. Incubate in the dark for 15 minutes at room temperature (20-25°C) or for 30 minutes at 4°C.The recommended negative control is an appropriate isotype control.
9. Add 2 mL of flow cytometry 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.

B. Procedure for preparation of RESTING PLATELETS

1. Centrifuge a tube of freshly drawn (Citrate) blood at 75xg for 20 minutes.
2. Remove platelets (top layer), and wash twice by adding 2 mL of wash solution and resuspend the cells. Mix well.
3. Transfer 5 µL of platelets to a 12 x 75 mm cytometer tube.
4. Add 95 µL of wash solution and mix well.
5. Add the appropriate volume of the antibody and mix gently with a vortex mixer. The optimal volume should be determined by the individual laboratory.
6. Incubate in the dark for 15 minutes at room temperature (20-25°C) or for 30 minutes at 4°C. The recommended negative control is an appropriate isotype control.
7. Add 2 mL of flow cytometry solution and resuspend the cells. Mix well.

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8. Centrifuge at 540xg for 5 minutes and carefully aspirate the supernatant so as not to touch the cell pellet.

9. Resuspend the pellet in 0.3 ml of flow cytometry solution.

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.
- Flow cytometry solution: 20 Mm NaH_2PO_4 , 150 NaCl, pH 7.2 + 1% Paraformaldehide.
- Phorbol 12-myristate 13-acetate (PMA) or thrombin solution
- Isotype control: http://immunostep.com/22-isotype-controls