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FcR blocking Reagent

Reference	Size
FCR	200 test
	400 test

PRODUCT DESCRIPTION

FcR Blocking is a reagent used to block certain non-specific binding of antibodies to receptors of constant fraction (FcR), increasing the specificity of antibodies in flow cytometry.

Recommended usage: This product has been designed to block receptors constant fraction of monocytes and macrophages to avoid nonspecific binding of antibodies and fluorochromes and increasing the affinity of the antibodies to their targets wich can complicate interpretation of flow cytometric results.

It is especially indicated when working with specific antigen markers or with fluorochromes such as cyanines.

Aplications

- Block the binding of antibodies to Fc receptors on human cells.
- Blocking of bead binding to Fc receptors in human cells

Presentation: liquid

Storage instructions: Store in the dark, refrigerated between 2 ºC and 8 ºC.

Reagent provided: FcR Blocking Reagent for human (10 µl/test) supplied in buffer containing protein stabilizer solution, PBS 20 mM and sodium azide 0,09%, pH 7,2.

Recommendations and warnings: The reagents contain sodium azide. In acid conditions, it is transformed into hydrazoic acid, a highly toxic compound.
Avoid microbial contamination of the reagent.

Protect from light.

Deviations from the recommended procedure could invalidate the analysis results.
For professional use only.
Reagents should not be used if any evidence of deterioration is observed. For more information, please contact our technical service: tech@immunostep.com

FcR Blocking Reagent protocol for indirect labelling of human cells

1. Transfer the sample of blood to a 12 x 75 mm polystyrene test tube (10^5 to 10^8 cells/test).
2. Add 10 µL of FcR Blocking Reagent (the optimal volume should be determined by the individual laboratory).
3. Mix well and incubate for 10 minutes at room temperature (20-25 °C).
4. Add purified antibodies according to manufacturer's recommendation and mix gently with a vortex mixer.
5. The recommended negative control is a non-reactive PU- antibody of the same isotype.
6. Incubate in the dark at room temperature at 4 °C for 30 minutes or at room temperature (20-25 °C) for 15 minutes.
7. Add 2 mL 0.01 mol/L PBS (It betters that it containing 2% bovine serum albumin) and resuspend the cells by using a vortex mixer.
8. A washing is made with centrifugation at 2000 rpm for 5 min in order to remove the McAb not bound to its antigen.
9. A secondary conjugated with some fluorochrome is added and the mixture is incubated at room temperature for 15 min in the darkness. The absence of light is necessary so that the fluorochrome will not deteriorate since it shows a high degree of photoinstability.
10. After the incubation period, an erythrocyte-lysing solution is added at the amount recommended by the manufacturer and the mixture is incubated at room temperature in the darkness (the blood should be well mixed with the lysing solution).
11. The tubes are centrifuged at 2000 rpm for 5 minutes. The supernatant is removed with a Pasteur pipette or with a vacuum pump.

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12. The cell pellet is resuspended and a final wash is made with 3-5 mL of PBS at 2000 rpm for 5 min.

13. After removing the supernatant and resuspending the cell pellet, some 300 µL of PBS is added and the readings on the flow cytometer are recorded.

14. Analyse on a flow cytometer or store at 2-8 °C in the dark until analysis. Samples can be run up to 24 hours after lysis.

FcR Blocking Reagent protocol for direct labelling of human cells

1. Transfer 100 µL of anticoagulated (EDTA) blood to a 12 x 75 mm polystyrene test tube (10^6 cells).

2. Add 10 µL of FcR Blocking Reagent (the optimal volume should be determined by the individual laboratory).

3. Mix well and incubate for 10 minutes at room temperature (20-25 °C).

4. Add antibodies according to manufacturer's recommendation and mix gently with a vortex mixer.

5. The recommended negative control is a non-reactive conjugated antibody of the same isotype.

6. Incubate in the dark at room temperature at 4 °C for 30 minutes or at room temperature (20-25 °C) for 15 minutes.

7. Add 1,5 mL of Lysing Solution to each sample and mix gently with a vortex mixer. Incubate for 10 minutes at room temperature in the dark.

8. Centrifuge at 1000 x g for 5 minutes. Gently aspirate the supernatant and discard it leaving approximately 50 µL of fluid.

9. Add 2 mL 0.01 mol/L PBS (It betters that it containing 2% bovine serum albumin) and resuspend the cells by using a vortex mixer.

10. Centrifuge at 1000 x g for 5 minutes. Gently aspirate the supernatant and discard it leaving approximately 50 µL of fluid.

11. Resuspend pellet in an appropriate fluid for flow cytometry, e.g. 0.3 mL PBS. The PBS should contain 1% paraformaldehyde (fixative) if samples are not analysed the same day.

12. Analyse on a flow cytometer or store at 2-8 °C in the dark until analysis. Samples can be run up to 24 hours after lysis.

WARRANTY

Warranted only to conform to the quantity and contents stated on the label or in the product labelling at the time of delivery to the customer. Immunostep disclaims hereby other warranties. Immunostep's sole liability is limited to either the replacement of the products or refund of the purchase price.

MANUFACTURED BY

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