FcR blocking Reagent

REF	Σ	
FCR	200 test 400 test	RUO

1. PRODUCT DESCRIPTION

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.

2. RECOMMENDED USAGE

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

Presentation: liquid.

Storage instruction: Shipped at ambient conditions, upon arrival store at 4°C.

3. REAGENTS PROVIDED

FCR Blocking Reagent contains sufficient volume per 200 or 400 test using 10 µl/test This reagent is supplied in buffer containing protein stabilizer solution, PBS 20 mM and sodium azide 0,09% as preservative, pH 7,2.

4. REAGENTS NO PROVIDED

- Wash solution: 20 Mm NaH2PO4, 150 NaCl, pH 7.2 + 0,09% Sodium azide (NaN3) + 0,5 % bovine serum albumin.
- 12x75mm Polystyrene Round Bottom Tubes (cytometer tubes).

5. RECOMMENDATION AND WARNINGS

This product contains sodium azide. In acid conditions, it is transformed into hydrazoic acid, a highly toxic compound. Azide compounds must be diluted in running water before being discarded. These conditions are recommended so as to avoid deposits in plumbing, where explosive conditions could develop.

Do not use after expiration date stamped on vial.

For professional use only.

Protect from light

6. PROTOCOL

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 100 ul (10^6 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 540xg for 5 min in order to remove the McAb not bound to its antigen.
9. Add a secondary conjugated reagent with some fluorochrome and mix. Incubate at room temperature for 15 min in the dark. The absence of light is necessary as the fluorochrome is photoinstability.
10. Centrifuge at 540xg for 5 minutes. Gently aspirate the supernatant and discard it leaving approximate
50 µL of fluid.
11. Resuspend and a made a final wash with 3-5 mL of PBS at 540xg for 5 min.
12. After removing the supernatant and resuspending the cell pellet, add 300 µL of PBS and adquire on the flow cytometer are recorded. 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. 7Add 1,5 mL of Lysing Solution to each sample and mix gently with a vortex mixer. Incubate for 10minutes at room temperature in the dark.
8. Centrifuge at 540 xg 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 540xg 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.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.

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)

7. 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.

8. ADDITIONAL INFORMATION

For research use only. Not for diagnostic use.

Not for resale. Immunostep will not be responsible of violations that may occur with the use of this product. Any use of this product other than the specified in this document is strictly prohibited.

Unless otherwise indicated by Immunostep by written authorization, this product is intended for research only and is not to be used for any other purpose, including without limitation, for human or animal diagnostic, therapeutic or commercial purposes.

Please, refer to www.immunostep.com technical support for more information.

9. EXPLANATION OF SYMBOLS

REF	Catalog reference
Σ	Contains sufficient for <n> test
	Regulatory Status
RUO	Research Use Only
	Manufacturer

10. MANUFACTURED BY:

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