Anti-Human CD16 (GRM1)

	REF	Σ		[A]	
PURE	16PU	1 mg	10 µL/test	1 mg/ml	RUO
FITC	16F-100T	100 test	20 µL/test	0,05 mg/ml	

1. PRODUCT DESCRIPTION

Clone: GRM1;
Isotype: IgG2a, kappa;
Tested application: flow cytometry;
Immunogen: The anti-CD16 monoclonal antibody derives from human polymorphonuclear leukocytes;
Species reactivity: Human;
Storage instruction: store in the dark at 2-8 °C;
Storage buffer: aqueous buffered solution containing protein stabilizer and 0.09% sodium azide (NaN_3);
Recommended usage: Mouse Anti-Human CD16 monoclonal antibody FITC-conjugated, clone GRM1, is recommended for use in flow cytometry for identification of FcgammaRIII antigen (FcγRIII) present on NK cells, neutrophils and macrophages in peripheral blood and bone marrow. This reagent is effective for direct immunofluorescence staining of human tissue for flow cytometric analysis using 1 test for 10^6 cells;
Presentation: liquid;
Source: Supernatant proceeding from an in vitro cell culture of a cell hybridoma;
Purification: Affinity chromatography;
Other names: FCRIII, Fc-gamma receptor III, CD16, FCG3, FCGR3, IGFR4;
Gene ID: 2214;
Molecular weight: Ig superfamily, transmembrane form (50-65 kDa) or GPI-linked form (48 kDa).

2. ANTIGEN DETAILS

Large description: The CD16 molecule has been described as the low affinity Fc receptor (FcRIII) for complexed IgG which may exist either as a transmembranous form (FcRIIIa) which is expressed on NK cells, and macrophages or as glycosylphosphatidylinositol (GPI)-anchored form (FcRIIIb) expressed on neutrophils. The GPI-anchored CD16B exists as two allelic forms termed NA1 (CD16BNA1) and NA2 (CD16BNA2).[4]

Clone GRM1 recognizes an epitope on the distal domain. It binds strongly with neutrophils of NA2 homozygotes and reacts weakly with neutrophils of NA1 homozygotes, while on neutrophils of NA1/NA2 heterozygotes it reacts with intermediate intensity(I). The mobility of the CD16-antigen is dependent on the NA1/NA2 allotype of the neutrophil donor[2].

Clone GRM1 recognizes NA2-FcγRIIIb and FcγRIIIa, whereas clone 3G8 is an Anti-pan FcγRIII[3].

3. 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.

4. ADDITIONAL INFORMATION

For research use only. Not for diagnostic use.

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5. PROTOCOL

- **Direct Immunofluorescence Cell Surface Staining Protocol**

1. Transfer 100 ul (106 cells/test) of the sample to a 12 x 75 mm polystyrene test tube.
2. Add the suggested volume indicated on the antibody vial to the 12x75 mm cytometer tube.
3. Mix well and incubate in the dark at room temperature at 4 °C for 30 minutes or at room temperature (20-25 °C) for 15 minutes.
4. After the incubation period, add 1,5 ml of an erythrocyte-lysing solution and Incubate at room temperature in the darkness (the blood should be well mixed with the lysing solution).
5. Centrifuge tubes at 540xg for 5 minutes. The supernatant is removed with a Pasteur pipette or with a vacuum pump.
6. Resuspend and wash with 3-5 mL of PBS at 540xg for 5 min.
7. After removing the supernatant and resuspending the cell pellet, add 300µL of PBS and adquire on the flow cytometer are recorded.
8. 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.

- **Indirect Immunofluorescence Cell Surface Staining Protocol**

1. Transfer 100 ul (106 cells/test) of the sample to a 12 x 75 mm polystyrene test tube
2. Add purified reagent according to manufacturer's recommendation and mix gently with a vortex mixer.
3. Incubate in the dark at room temperature at 4 °C for 30 minutes or at room temperature (20-25 °C) for 15 minutes.
4. 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. Centrifuge at 540xg for 5 min in order to remove the McAb not bound to its antigen.
5. 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.
6. After the incubation period, add 1,5 ml of an erythrocyte-lysing solution and Incubate at room temperature in the darkness (the blood should be well mixed with the lysing solution). Centrifuge at 540xg for 5 minutes. The supernatant is removed with a Pasteur pipette or with a vacuum pump.
7. Resuspend and a made a final wash with 3-5 mL of PBS at 540xg for 5 min.
8. After removing the supernatant and resuspending the cell pellet, add 300 µL of PBS and adquire on the flow cytometer are recorded.
9. 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.

6. REFERENCES

1. Claudio Ortolani. Flow Cyometry of Hematological. John Wiley & Sons, 15 ago. 2011.
2. Ruiz-Cabello F, Lopez Nevot MA, Garrido A, Garrido F. A study of GRM1 monoclonal antibody that reacts with natural killer cells and granulocytes. Nat Immun Cell Growth Regul1987; 6(2):99-108.
3. 3Tamm A, Schmidt RE. The binding epitopes of human CD16 (Fc gamma RIII) monoclonal antibodies. Implications for ligand binding. J Immunol1996 Aug 15;157(4):1576-81.
4. Harry R. Koene, Marion Kleijer, Dirk Roos, Masja de Haas and Albert E. G. Kr Von dem Borne. FcγRIIIB Gene Duplication: Evidence for Presence and Expression of Three Distinct FcgRIIIB Genes in NA(1+,2+)SH(+) Individuals.

7. EXPLANATION OF SYMBOLS

	Form
REF	Catalog reference
Σ	Contains sufficient for > test
	Quantity per test
	Regulatory Status
RUO	Research Use Only
[A]	Concentration
	Manufacturer

8. MANUFACTURED BY:

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