

Anti-Human CD64 (10.1)



PURE	64PU-OIMG	100 µg	1 mg/ml	
FITC	64F-100T	100 test	20 µL/test	2 mg/ml
PE	64PE-100T	100 test	20 µL/test	2 mg/ml
APC	64A-100T	100 test	5 µL/test	0,05 mg/ml
PerCP	64PP-OIMG	100 µg	5 µL/test	0,05 mg/ml

RUO

5. PROTOCOL

■ Direct Immunofluorescence Cell Surface Staining Protocol

1. Transfer 100 µl (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 mix. 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 300 µL of PBS and acquire 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 µl (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 mix. 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 acquire 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. Dougherty GJ, Selvendran Y, Murdoch S, Palmer DG, Hogg N. The human mononuclear phagocyte high-affinity Fc receptor, FcRI, defined by a monoclonal antibody, 10.1. Eur J Immunol1987 Oct;17(10):1453-9.
2. Indik ZK, Hunter S, Huang MM, Pan XQ, Chien P, Kelly C, et al. The high affinity Fc gamma receptor (CD64) induces phagocytosis in the absence of its cytoplasmic domain: the gamma subunit of Fc gamma RIIIA imparts phagocytic function to Fc gamma RI. Exp Hematol1994 Jul;22(7):599-606.
3. Hashimoto S, Yamada M, Motoyoshi K, Akagawa KS. Enhancement of macrophage colony-stimulating factor-induced growth and differentiation of human monocytes by interleukin-10. Blood1997 Jan 01;89(1):315-21.
4. Sanchez-Torres C, Garcia-Romo GS, Cornejo-Cortes MA, Rivas-Carvalho A, Sanchez-Schmitz G. CD16+ and CD16- human blood monocyte subsets differentiate in vitro to dendritic cells with different abilities to stimulate CD4+ T cells. Int Immunol2001 Dec;13(12):1571-81.

7. EXPLANATION OF SYMBOLS

	Form
	Catalog reference
	Contains sufficient for > test
	Quantity per test
	Regulatory Status
	Research Use Only
	Concentration
	Manufacturer

8. MANUFACTURED BY:

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1. PRODUCT DESCRIPTION

Clone: 10.1;
Isotype: IgG1;
Tested application: flow cytometry;
Immunogen: The anti-CD64 monoclonal antibody derives from human rheumatoid synovial fluid cells and fibronectin-purified monocytes;
Species reactivity: Human, Cross-Reactivity: Chimpanzee, Baboon, Cynomolgus, Rhesus, Capuchin Monkey, Squirrel Monkey;
Storage instruction: store in the dark at 2-8 °C;
Storage buffer: aqueous buffered solution containing protein stabilizer and 0.09% sodium azide (NaN₃);
Recommended usage: Immunostep's CD64, clone 10.1 is a monoclonal antibody intended for the identification and enumeration of monocytes, macrophages, dendritic cells, granulocytes activated with interferon-gamma and early myeloid lineage cells using flow cytometry. This reagent is effective for direct immunofluorescence staining of human tissue for flow cytometric analysis using 1 test for 10⁶ cells.
Presentation: liquid;
Source: Supernatant proceeding from an in vitro cell culture of a cell hybridoma;
Purification: Affinity chromatography;
Other names: High affinity immunoglobulin gamma Fc receptor I, IgG Fc receptor I, Fc-gamma RI, FcRI, Fc-gamma RIA, Fc-gammaRIa;
Gene ID: 2209;
Molecular weight: 75 kDa.

2. ANTIGEN DETAILS

Large description: This antibody reacts with the CD64-antigen (FcRI), a 75 kDa type I transmembrane protein. CD64 is the high affinity receptor for IgG and is involved in antibody-dependent cell-mediated cytotoxicity (ADCC), phagocytosis, and regulation of cytokine production.⁽¹⁻⁴⁾

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