

Anti-Human CD14 (47-3D6)

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PURE	14PU	1 mg	10 µL/test	1 mg/ml	
APC-750	14AC750-100T	100 test	5 µL/test	0,2 mg/ml	

1. PRODUCT DESCRIPTION

Clone: 47-3D6;
Isotype: IgG2a;
Tested application: flow cytometry;
Immunogen: The anti-CD14 monoclonal antibody derives from native purified CD14 cells from human lung Angiotensin converting enzyme;
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 (NaN₃);
Recommended usage: Immunostep's CD14, clone 47-3D6 is a monoclonal antibody intended for the identification and enumeration of human monocytes 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: Monocyte differentiation antigen CD14, Myeloid cell-specific leucine-rich glycoprotein;
Gene ID: 929;
Molecular weight: 55 kDa.

2. ANTIGEN DETAILS

Large description: The monoclonal antibody is directed against the CD14 antigen, which is expressed on human monocytes and macrophages. The antibody reacts with human monocytes and macrophages; weak reactions may occur with neutrophils.^[6-7]

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.

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.

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 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 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 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. Almeida J, Bueno C, Alguero MC, Sanchez ML, de Santiago M, Escribano L, et al. Comparative analysis of the morphological, cytochemical, immunophenotypical, and functional characteristics of normal human peripheral blood lineage(-)/CD16(+)/HLA-DR(+)/CD14(-/lo) cells, CD14(+) monocytes, and CD16(-) dendritic cells. Clin Immunol2001 Sep;100(3):325-38.
2. Chomarat P, Dantin C, Bennett L, Banchereau J, Palucka AK. TNF skews monocyte differentiation from macrophages to dendritic cells. J. Immunol 2003 Sep1;17(5):2262-9.
3. Haziot A, Tsuberi BZ, Goyert SM. Neutrophil CD14: biochemical properties and role in the secretion of tumor necrosis factor-alpha in response to lipopolysaccharide. J Immunol1993 Jun 15;150(12):5556-65.
4. Knapp W. Leucocyte typing IV : white cell differentiation antigens. Oxford: Oxford University Press; 1989.
5. Li G, Kim YJ, Mantel C, Broxmeyer HE. P-selectin enhances generation of CD14+CD16+ dendritic-like cells and inhibits macrophage maturation from human peripheral blood monocytes. J Immunol2003 Jul 15;171(2):669-77.
6. Scherberich JE, Nockher WA. CD14++ monocytes, CD14+/CD16+ subset and soluble CD14 as biological markers of inflammatory systemic diseases and monitoring immunosuppressive therapy. Clin Chem Lab Med1999 Mar;37(3):209-13.
7. Schlossman SF. Leucocyte typing V : white cell differentiation antigens : proceedings of the Fifth International Workshop and Conference : held in Boston, USA, 3-7 November, 1993. Oxford: Oxford University Press; 1995.

7. EXPLANATION OF SYMBOLS

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

8. MANUFACTURED BY:



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