Anti-Human CD14 (47-3D6)

Fluorochrome	Reference	Test
FITC	14F-100T	100 test
PE	14PE-100T	100 test
APC	14A-100T	100 test

IVD CE immunostep we know how

PRODUCT DESCRIPTION

Other Names: Monocyte differentiation antigen CD14, Myeloid cell-specific leucine-rich glycoprotein
Description: The anti-CD14 monoclonal antibody derives from native purified CD14 cells from human lung Angiotensin converting enzyme.
Clone: 47-3D6
Isotype: Mouse IgG2a, kappa
Reactivity: Human
Source: Supernatant proceeding from an *in vitro* cell culture of a cell hybridoma.
Purification: Affinity chromatography.
Compositión: Mouse anti-human CD14 monoclonal antibody conjugated with a fluorochrome and in an aqueous solution which contains stabilising protein and 0.09% sodium azide (NaN_3).

Fluorochrome	Reagent provided	Concentration (µg/ml)
FITC (Fluorescein isothiocyanate)	100 ug in 2 ml	50
PE (R-Phycoerythrin)	25 ug in 2 ml	12,5
APC (Allophycocyanin)	25 ug in 2 ml	12,5

RECOMMENDED USAGE

Immunostep's CD14, clone 47-3D6, is a monoclonal antibody intended for *in vitro* diagnostic use in the identification and enumeration of human sample monocytes that express CD14 using flow cytometry.

CLINICAL RELEVANCE

The Immunostep CD14 monoclonal antibody can be used for the required for induction of cytokines and/or lethality in murine model of shock induced endotoxin or live E. Coli, soluble forms of CD14 are found in plasma at a concentration of about 3µg/ml, and, in whole blood, the amount of soluble CD14 exceeds the amount of membrane-bound CD14 by 2-3 logs[1-6].

PRINCIPLES OF THE TEST

The anti-CD14 monoclonal antibody binds to the surface of cells that express the CD14 antigen. To identify these cells, the sample is incubated with the antibody and is analysed by flow cytometry.

APPROPRIATE STORAGE AND HANDLING CONDITIONS

Store in the dark, refrigerated between 2 °C and 8 °C. DO NOT FREEZE. The antibody is stable until the expiry date stated on the vial label if kept at 2°C-8°C. Do not use after the date indicated.

Once the vial is open, the product is stable for 90 days.

EVIDENCE OF DETERIORATION

Reagents should not be used if any evidence of deterioration is observed. For more information, please contact our technical service: tech@immunostep.com

The product's normal appearance is a semi-transparent, colourless liquid. It should not be used if liquid medium is cloudy or contains precipitate. It should be odourless.

RECOMMENDATIONS AND WARNINGS ⚠

a) The reagents contain 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. The safety data sheet (SDS) is available online at www.immunostep.com
b) Avoid microbial contamination of the reagent.
c) Protect from light. Use dim light during handling, incubation with cells and prior to analysis.
d) Never mouth pipette.
e) In the case of contact with skin, wash in plenty of water.
f) The samples should be handled in the same way as those capable of transmitting infection. Appropriate handling procedures should be guaranteed.
g) Do not use after the expiry date indicated on the vial.
h) Deviations from the recommended procedure could invalidate the analysis results.
i) FOR *IN VITRO* DIAGNOSTIC USE.
j) For professional use only.
k) Before acquiring the samples, it is necessary to make sure that the flow cytometer is calibrated and compensated.

SAMPLE COLLECTION

The extraction of venous blood samples should be carried out in blood collection tubes using the appropriate anticoagulant (EDTA or heparin)[7,8]. For optimum results, the sample should be processed during the six hours following the extraction. Samples which cannot be processed within the 48 hours following the extraction should be discarded.

MATERIALS REQUIRED BUT NOT PROVIDED

- Isotype controls:

Fluorochrome	Isotype control	Immunostep Reference
FITC	Mouse IgG2a	ICIGG2AF-100UG
PE		ICIGG2APE-50UG
APC		ICIGG2AA-50UG

- Centrifuge
- Commonly used 12 x 75-mm flow cytometry assay tubes
- Micropipettes for dispensing volumes from 5 µl to 2 ml
- Blood collection tubes with anticoagulant.
- Phosphate buffered saline (PBS) with 0.09% sodium azide. It is recommendable to add 0.5% BSA
- Vacuum system
- Lysing solution
- Flow cytometer equipped with laser and appropriate fluorochrome filters
- Vortex Agitator

SAMPLE PREPARATION:

1. Add the suggested volume indicated on the antibody vial to a 12x75-mm cytometer tube. It is advisable to prepare an additional tube with the appropriate isotype control *(please see materials required but not provided).*
2. Add 100 µL of sample (up to 10^6 cells) and mix properly in the vortex.
3. Incubate in the dark for 15 minutes at room temperature (20-25°C) or for 30 minutes at 4°C.
4. Add 2 ml of the lysing solution, mix in the vortex and incubate in the dark for 10 minutes or until the sample is lysed.
5. Centrifuge at 540g for five minutes and carefully withdraw the supernatant by suction so as not to touch the cell pellet. Leave 50 µl of non-aspirated liquid.
6. Resuspend pellet.
7. Add 2 ml of PBS *(please see materials required but not provided).*
8. Centrifuge at 540g for five minutes and carefully withdraw the supernatant by suction so as not to touch the cell pellet. Leave 50 µl of non-aspirated liquid.
9. Resuspend the pellet in 0.3 ml of PBS.

Acquire on a flow cytometer or store in the dark at 2°C -8°C until the analysis is carried out. Samples should be acquired within the 3 hour after lysis.

FLOW CYTOMETRY ANALYSIS

Collect the fluorescence attributed to monoclonal antibody CD14 and determine the percentage of stainend cells.

It is necessary to use an isotype control conjugated with the same fluorochrome, of the same type of immunoglobulin heavy chain and concentration as that of the CD14, so as to evaluate and correct the unspecific binding of leucocytes *(please see materials required but not provided).* Set an analysis region to eliminate fluorescence background noise and to include positively stained cells.

Below is an example diagram of stained cells:

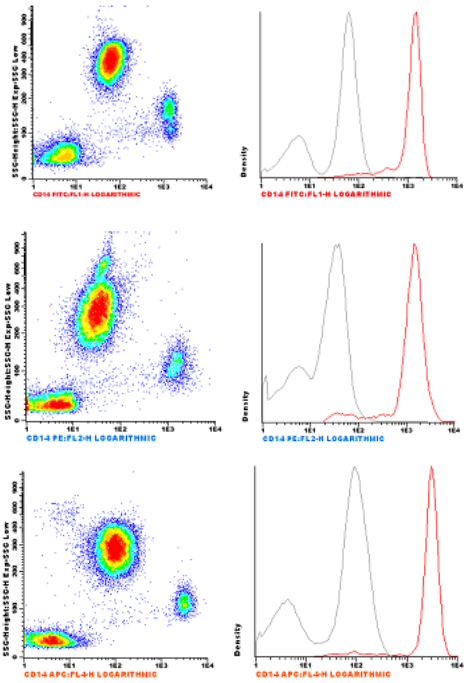

Fig. 1: On the left, a biparametric diagram of the average fluorescence intensity of peripheral blood stained with CD14+ and its internal complexity (SSC). Right, a diagram of the same specimen in histogram format.

LIMITATIONS OF THE PROCEDURE

1. Incubation of antibody with cells for other than the recommended procedures may result in a reduction or loss of antigenic determinants from the cell surface.
2. The values obtained from normal individuals may vary from laboratory to laboratory; it is therefore suggested that each laboratory should establish its own normal reference range.
3. Abnormal cells or cell lines may show a higher antigen density than normal cells. In some cases, this could require the use of a greater quantity of monoclonal antibody than is indicated in the procedures for sample preparation.
4. In whole blood samples, red blood cells found in abnormal samples, as well as nucleated red cells (from both normal and abnormal specimens) may be resistant to lysis. Longer periods of red blood cell lysing may be needed in order to avoid the inclusion of unlysed cells in the lymphocyte gated region.
5. Blood samples should not be refrigerated for an extensive period (more than 24 hours), since the number of viable cells will gradually decrease, and this may have an effect on the analysis. In order to obtain the best values, they should be
6. kept at room temperature immediately prior to incubation with the monoclonal antibody.
7. Accurate results with flow cytometric procedures depend on correct alignment and calibration of the lasers, as well as correct gate settings.

REFERENCE VALUES

Abnormal results in the percentage of cells expressing the antigen or in its levels of expression may be due to pathological conditions.

It is advisable to know the normal antigen expression patterns in order to ensure a proper interpretation of the results[69,10,11]
The values obtained from healthy individuals may vary from laboratory to laboratory; it is therefore suggested that each laboratory should establish its own normal reference range.

CHARACTERISTICS

SPECIFICITY

Blood samples were obtained from Caucasian healthy normal donors and were stained with Immunostep CD14 monoclonal antibody. Cells contained in the lymphocyte, monocyte and granulocyte regions were selected for analysis. Blood samples were processed by a direct immunofluorescence staining for flow cytometric analysis protocol.
To evaluate the reagent's Specificity (cross-reactivity with other cell populations), 10 blood samples (5 for FITC) from healthy donors were studied, stained with an adequate isotype control and the MAb to study. The percentage of lymphocytes, monocytes and granulocytes stained with the mentioned MAb was evaluated. The results obtained are shown in the following table:

FITC		Lymphocytes	Monocytes	Granulocytes
N	Valid	5	5	5
	Missing	0	0	0
Mean		2,34	77,82	64,40
Median		1,83	86,72	58,81
Mode		1,39 (a)	31,51(a)	41,09 (a)
Std. Deviation		1,22	26,31	17,94
Variance		1,49	692,28	322,01
Range		3,07	65,20	46,28
PE		Lymphocytes	Monocytes	Granulocytes
N	Valid	10	10	10
	Missing	0	0	0
Mean		10,43	97,03	99,33
Median		9,53	98,83	99,79
Mode		6,55(a)	90,43(a)	96,52(a)
Std. Deviation		2,81	3,34	1,08
Variance		7,93	11,16	1,17
Range		8,45	9,32	3,46
APC		Lymphocytes	Monocytes	Granulocytes
N	Valid	10	10	10
	Missing	0	0	0
Mean		23,60	96,92	85,14
Median		27,41	100,00	88,66
Mode		6,12 (a)	100,00(a)	53,11(a)
Std. Deviation		11,65	6,85	13,14
Variance		135,76	47,04	172,66
Range		33,21	21,90	45,11

a. Multiple modes exist. The smallest value is shown

SENSIBILITY

Sensitivity of the Immunostep CD14 monoclonal antibodies was determined by staining a blood sample from donor. Dilutions of a peripheral blood sample were made to check the concentration scale of stained cells obtained. The results show an excellent correlation level between the results obtained and expected based on the dilution used.

To determine the consistency of the conjugated monoclonal antibody as opposed to small variations (but deliberate). It provides an indication of its reliability during its normal use.

FITC	R	R Square	Adjusted R Spuare	Std. Error of the Estimate
	0,996 (a)	0,992	0,991	2,18835
PE	R	R Square	Adjusted R Spuare	Std. Error of the Estimate
	0,999(a)	0,998	0,998	1,21148
APC	R	R Square	Adjusted R Spuare	Std. Error of the Estimate
	0,995(a)	0,990	0,989	2,95353

(a) Predictors: (Constant), Obtained

REPRODUCIBILITY

Reproducibility for the Immunostep CD14 -conjugated monoclonal antibodies was determined by performing 10 replicated determinations of each antibody in each of three CD14+ ranges, high, medium and low. Thus, a total of 30 determinations were performed for each form of CD14. In this manner, reproducibility was demonstrated throughout the entire measuring range.
The 10 determinations for each range were performed by the staining, processing and analysis of 10 separate samples. Monocytes were selected for the analysis of percent cells stained in each of the three ranges.
To perform this study, anticoagulated blood was obtained from a normal donor expressing a high percentage of CD14+ cells. Mid-range and low range samples were obtained by mixing known CD14- cells in appropriate ratios, while maintaining the same total cell concentration for the three ranges.

The study was performed in each of three independent laboratories, in the manner that each laboratory obtained, stained and analyzed separate blood samples.

FITC	N	Minimum	Maximum	Mean	Std. Deviation
High	10	9,56	10,60	9,91	0,32
Medium	10	5,40	6,10	5,60	0,19
Low	10	2,74	2,98	2,89	0,06
Valid N (listwise)	10				
PE	N	Minimum	Maximum	Mean	Std. Deviation
High	10	81,58	87,85	85,56	2,06
Medium	10	60,96	69,75	66,33	2,54
Low	10	14,75	16,80	15,85	0,74
Valid N (listwise)	10				

APC	N	Minimum	Maximum	Mean	Std. Deviation
High	10	77,46	80,30	79,08	0,81
Medium	10	56,80	59,30	57,88	0,87
Low	10	45,20	58,67	50,59	4,022
Valid N (listwise)	10				

*Note: Data analyzed with SPSS for Windows 11.0.1

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.

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