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Anti- Human CD5 (L17F12)

Fluorochrome	Reference	Test
APC	5A-100T	100 test

IVD

PRODUCT DESCRIPTION

Other Names: T-cell surface glycoprotein CD5, Lymphocyte antigen T1/Leu-1, Leu-1, Ly-1, T1, Tp67.
Description: The anti-CD5 monoclonal antibody derives from the Human acute lymphoblastic leukemia (ALL) T cells. The antibody is formed by an IgG2a heavy chain and a kappa light chain.
Clone: L17F12
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 CD5 monoclonal antibody conjugated with a fluorochrome and in an aqueous solution which contains stabilising protein and 0.09% sodium azide (NaN3).

Fluorochrome	Reagent provided	Concentration (µg/ml)
APC (Allophycocyanin)	20 ug in 2 ml	10

RECOMMENDED USAGE

Immunostep's CD5, clone L17F12, is a monoclonal antibody intended for *in vitro* diagnostic use in the identification and enumeration of human sample lymphocytes that express CD5 using flow cytometry.

CLINICAL RELEVANCE

The Immunostep CD5 monoclonal antibody may also be used, in combination with other makers, for diagnosis or prognosis such as a phenotypic marker for some B-cell lymphoproliferative disorders (B-CLL, mantle zone lymphoma, Hairy cell leukemia, etc...).

The CD5+ population is expanded in some autoimmune disorders (Rheumatoid Arthritis, Sjögren Syndrome, Insulin-dependent Diabetes Mellitus, graves disease, etc...) and in vivo, down-modulation of CD5 by mAbs induces T cell unresponsiveness, and is reported to prevent experimental autoimmune encephalomyelitis in the rat.

Anti-CD5 antibody treatment has a partial therapeutic effect on collagen type II-induced arthritis in (DBA/1) mice and on GVHD after allogeneic marrow transplantation in humans.

The frequency of CD3+ T cells that lack expression of CD5 is dramatically increased following bone marrow transplantation and correlates with the presence of GVHD.

Finally, Herpes virus infections induce loss of CD5 expression (and other costimulatory molecules, like CD28 and CD6) in the expanded CD8+ human T cells.

PRINCIPLES OF THE TEST

The anti-CD5 monoclonal antibody binds to the surface of cells that express the CD5 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)[2,3].

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
APC	Mouse IgG2a	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 CD5 and determine the percentage of stainend cells. It is necessary to use an isotope control conjugated with the same fluorochrome, of the same type of immunoglobulin heavy chain and concentration as that of the CD5, so as to evaluate and correct the unspecific binding of lymphocytes *(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 peripheral blood stained:

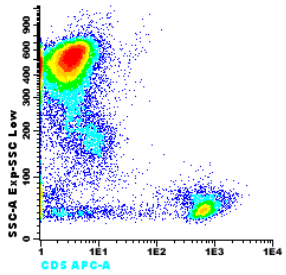

Fig. 1: A biparametric diagram of the average fluorescence intensity of the CD5+ lymphocyte population and its internal complexity (SSC) in a peripheral blood specimen from a healthy donor.

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 kept at room temperature immediately prior to incubation with the monoclonal antibody.
6. 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[4,5,6].

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 healthy normal donors of Caucasian were stained with Immunostep CD5 APC monoclonal antibody.
Cells contained in the lymphocyte, monocyte and granulocyte regions were selected for analysis. Blood samples were processed by a leukocyte method, with a direct immunofluorescence staining for flow cytometric analysis.

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
% Isotype control	10	,14	2,66	,9450	,86341
% Platelets	10	,02	1,13	,2980	,38583
% Erythrocytes	10	,00	,32	,0800	,09250
% Monocytes	10	,00	,37	,1090	,11846
% Neutrophils	10	,00	1,38	,2700	,46442
Valid N (listwise)	10				

LINEARITY

Linearity of the Immunostep CD5 APC was determined by staining T lymphocytes (positive population) and Neutrophils (negative population) cells previously separated on a FACSAria II from a human donor. Different dilutions of both of samples were made to determine the consistency of the conjugated monoclonal antibody as opposed to small variations (but deliberate) and 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. It provides an indication of its reliability during its normal use.

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Linear regression
1	,995 (a)	,990	,988	3,87690	Y= 1,044X – 1,578

(a)Predictors: (Constant), Obtained

REPETEABILITY

Repeatability or Intra-laboratory Reproducibility (Within-Laboratory Precision) for the Immunostep CD5 APC-conjugated monoclonal antibodies was determined according Clinical and Laboratory Standards Institute document EP05-A3 and ISO 5725 by performing 10 replicated determinations of 10 anticoagulated blood samples from healtly donors of different lymphocyte ranges and with 3 different batch.
Thus, a total of 300 determinations were performed to calculate the Repeatability.

	Between-Run Precission		Between-Lot Precission	
Parameter	SD	% CV	SD	% CV
MFI	178,27	2,51	412,19	5,53
% Positive cells	0,34	4,51	0,61	7,6

REPRODUCIBILITY

Reproducibility or Inter-laboratory Precissión for the Immunostep CD5 APC-conjugated monoclonal antibodies was determined according Clinical and Laboratory Standards Institute document EP05-A3 and ISO 5725 by performing 5 replicated determinations of 5 anticoagulated blood samples from healtly donors collected into Cyto-Chex BCT tubes and adquired over 5 days in three different laboratories with different flow cytometers: a FACSAriaII, a FACSCalibur and a FACSAccury C6.

Thus, a total of 375 determinations were performed to calculate the Reproducibility.

	Between-Day Precission		Between-Laboratory Precission	
Parameter	SD	% CV	SD	% CV
% Positive cells	0,27	3,63	0,05	0,66

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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