

Anti- Human CD45 (D3/9)

Fluorochrome	Reference	Test
FITC	45FI-100T	100 test
PE	45PEI-100T	100 test
PerCP	45PPI-100T	100 test
APC	45AI-100T	100 test



PRODUCT DESCRIPTION

Other Names: LCA, T200

Description: The anti-CD45 monoclonal antibody derives from the hybridisation of mouse myeloma cells and T cells from leukemic HPB-ALL. The antibody is formed by an IgG1 heavy chain and a kappa light chain.

Intended use: CD45 fluorochrome conjugated, is a single colour immunofluorescence reagent intended for identification of lymphoid and myeloid cell line, both bone marrow and peripheral blood of normal and pathological samples on a flow cytometer.

Clone: D3/9

HLDA: 4th International Workshops on Human Leucocyte Differentiation, WS Code 825.

Isotype: Mouse IgG1, kappa

Reactivity: Human

Source: Supernatant proceeding from an *in vitro* culture of a cell hybridoma or mouse ascites.

Purification: Affinity chromatography.

Composition: Mouse anti-human CD45 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)
FITC (Fluorescein isothiocyanate)	100 ug in 2 ml	50
PE (R-Phycoerythrin)	50 ug in 2 ml	25
PerCP (Peridin-cholophyll-protein complex)	100 ug in 2 ml	50
APC (Allophycocyanin)	50 ug in 2 ml	25

RECOMMENDED USAGE

Immunostep's CD45, clone D3/9, is a monoclonal antibody intended for *in vitro* diagnostic use in the identification and enumeration of human sample granulocyte, NK cells, lymphocytes and macrophages by flow cytometry that express CD45.

CLINICAL RELEVANCE

CD45 is a critical requirement for T and B cell antigen receptor-mediated activation and possible requirement for receptor-mediated activation in other leukocytes.

This reagent can be used in the characterization studies for immunophenotyping of leucocytes, which are widely applied in the characterization and follow-up of immunodeficiencies, autoimmune diseases, leukemias, etc

CD45 antibody has been used in immunohistochemistry to study the effects of the natural plasma constituent recovered from type 2 diabetic patients (dm-LDL) on endothelial cells and to study the expression, localization, and functional activity of TLIA in inflammatory bowel disease.

Anti-CD45 antibody can be used for study the expression of antimicrobial peptides and lysozyme in colonic epithelial cells of patients with ulcerative colitis.

Detection of distinct isoforms can distinguish between naive T cells and memory T cells, which is of interest in patients with immunodeficiency and autoimmune diseases.

Combination of CD45 with CD14 antibodies in the analysis of blood or bone marrow samples by flow cytometry shows variable expression of these antigens on different cell populations studies on the function of individual CD45 with potent immunosuppressive activity, suggesting that CD45 may be a useful target for drug design⁽¹⁻⁵⁾

PRINCIPLES OF THE TEST

The anti-CD45 monoclonal antibody binds to the surface of cells that express the CD45 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

- 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.
- l) In case of background, centrifuge at 2000 rpm for 2 minutes to avoid interferences.

SAMPLE COLLECTION

The extraction of venous blood samples should be carried out in blood collection tubes using the appropriate anticoagulant (EDTA or heparin)⁶⁻⁷. 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 IgG1	ICIGGIF-100UG
PE		ICIGGIPE-50UG
PerCP		ICIGGIPP-100UG
APC		ICIGGIA-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 10⁶ 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 CD45 and determine the percentage of stained 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 CD45, 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 stained peripheral blood from a healthy donor:

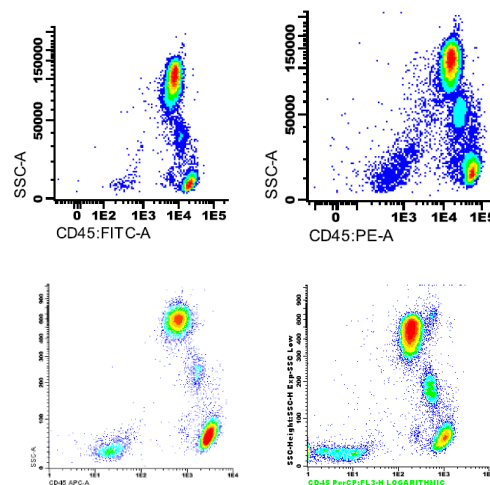


Fig. 1: Above, a biparametric diagram of the average fluorescence intensity of the CD45+ positive cells and its internal complexity (SSC) in a peripheral blood specimen from a healthy donor.

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.
- 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 un lysed cells in the lymphocyte gated region.
 - 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.
 - 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 results⁷⁻¹⁰

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

The anti-CD45 antibody, clone D3/9, was included in the 4th International Workshops on Human Leucocyte Differentiation, WS Code 825.

The antibody recognizes all isoforms of human CD45 antigen (Leukocyte Common Antigen), a transmembrane single protein chain type I expressed in high level in all cells of hematological origin, except in erythrocytes and platelets.

LINEARITY

Sensitivity of the Immunostep CD45 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.

Model	R	R Square	Adjusted R ²
FITC	,997(a)	,993	,63659
PE	,997(a)	,994	2,74825
PerCP	,990(a)	,979	5,12607
APC	,976(a)	,953	7,79558

a Predictors: (Constant), Expected

REPRODUCIBILITY

Reproducibility for the Immunostep CD45 conjugated monoclonal antibodies was determined by performing 10 replicated determinations of each antibody in each of three CD45+ ranges, high, medium and low. Thus, a total of 30 determinations were performed for each form of CD45. 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. Lymphocytes 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 CD45+ cells. Mid-range and low range samples were obtained by mixing known CD45- 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	82,87	88,82	86,9900	1,86322
Medium	10	62,56	66,61	64,6730	1,49411
Low	10	18,33	20,26	19,3490	,69223
Valid N (listwise)	10				
PE					
High	10	88,30	95,56	93,7650	2,04913
Medium	10	86,44	89,30	87,6350	,78801
Low	10	82,00	85,90	83,7820	1,51770
Valid N (listwise)	10				
PerCP					
High	10	97,22	99,14	98,7110	,54378
Medium	10	86,24	92,78	89,2460	2,06855
Low	10	78,42	89,38	82,2600	3,97419
Valid N (listwise)	10				
APC					
High	10	29,21	31,01	29,8260	,60768
Medium	10	18,44	19,74	19,1710	,47985
Low	10	5,14	6,44	5,7760	,40574
Valid N (listwise)	10				

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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