Anti- Human CD45 (HI30)

Fluorochrome	Reference	Test
OC515	450C2-100T	100 test

PRODUCT DESCRIPTION

Other Names: LCA, T200
Description: The anti-CD45 monoclonal antibody derives from the hybridisation of mouse myeloma cells and spleen cells from mice immunised with mononuclear cells from human peripheral blood and tonsil. The antibody is formed by an IgG1 heavy chain and a kappa light chain.
Intended use: CD45 fluorochrome conjugated, is a single color 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: HI30
HLDA: The anti-CD45 antibody, clone HI30, was included in the 4th International Workshops on Human Leucocyte Differentiation, WS Code N816[1].
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)
OC515	250 ug in 0,5 ml	500

RECOMMENDED USAGE

Immunostep's CD45, clone HI30, 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

This marker may be used on its own or in combination with other markers for the diagnosis or prognosis of some immunodeficiency diseases, autoimmune diseases, leukemias...

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

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.
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)[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
OC515	Mouse IgG1	ICIGG1OC-100UG

- 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 CD45 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 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:

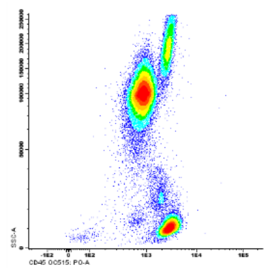

Fig. 1: Above, a biparametric diagram of the average fluorescence intensity of the CD45+ positive cells and its internal complexity (SSC) in a peripheral blood specimen from a healthy donor.

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.
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 un lysed 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

The anti-CD45 antibody, clone HI30, was included in the Fourth Workshop on Human Leukocyte Differentiation Antigens (HDLA), using Code N816[1].

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.

In order to analyze the analytical specificity, we determine the percentage of staining leukocytes, platelets and erythrocytes, identifying these populations with specific line markers. The protocol followed is the labeling of membrane antigens with erythrocyte lysis and subsequent washing.

The results obtained are shown in the following table:

Descriptive statistics

	Mín	Max	Media	typical deviation
% Leucocitos	99,80	99,99	99,9050	0,0675
% Plaquetas	0,00	0,17	0,0680	0,06125
% Eritrocitos	0,01	0,08	0,0370	,02497
Validos N	10			

LINEARITY

For the linearity analysis, different dilutions of a positive population and a negative population were carried out, keeping the total number of cells constant, and the relation between the expected percentages and those obtained was analysed.

The results obtained are shown in the following table:

R	R Square	Lineal Regresion
0,996	0.933	Y=0,7957X + 24,27

REPEATABILITY

The repeatability[9] of CD45, clone HI30, monoclonal antibody was established by performing 2 replicates during 10 days with a commercial control sample CD-Chex Plus (Streck, ref.213326).
Samples were analysed in a FACSAria III cytometer.

To calculate repeatability, the percentage of positive cells and the fluorescence intensity mean was analyzed.

The results obtained are shown in the following table:

Repeatability			
	Analysed parameter	typical deviation	% CV
OC515	IMF	17,32	1,98
	% positive cells	0,08516	0,08

REPRODUCIBILITY

In order to demonstrate reproducibility and inter-laboratory precision, 3 different lots of antibody were analyzed for 5 followed days by testing 2 replicates of a commercial control sample CD-Chex Plus (Streck, ref. 213326). This makes a total of 60 tests to analyze product reproducibility and accuracy between lotes[8]. Samples were acquired in two different laboratories.

To calculate reproducibility, percentage of positive cells was analyzed.

The test results are shown in the following grid:

	Precisión inter lots		**Precision inter laboratories**		**Precision**	
Fluorochrome	typical deviation	**% CV**	typical deviation	**% CV**	typical deviation	**% CV**
OC515	0,082	0,08	0,057	0,06	0,205	0,21

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.

REFERENCES

1. Knapp W, Dörken B, Gilks WR, et al, ed. Leucocyte Typing IV. New York, NY: Oxford University Press; 1989: 1-1182.
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