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Product: OC515 Anti-human CD45
Cat. Ref: 45OC2-100T
Reagent provided: 100 tests (5 µl / test)
Description: Monoclonal Mouse Anti-Human CD45 OC515, is recommended for use in flow cytometry for identification and analysis of CD45+ cells. The conjugate is provided in aqueous buffered solution containing protein stabilizer, and ≤0.09% sodium Azide
Clone: HI30
Isotype: IgG1
HLDA: 4th International Workshops on Human Leucocyte Differentiation, WS Code 816.
Fluorochrome: OC515 (Abs/Em Max: 405/518 nm)
Direct replacement for: Pacific Orange™and AmCyan

INTENDED PURPOSE.

CD45 OC515 is a monoclonal antibody conjugated that may be used to identification of lymphocytes, monocytes, granulocytes, thymocytes and malignant T and B cells. This antibody recognizes the CD45 antigen also known as Leukocyte Common Antigen or LCA. CD45 is expressed on all cells of hematopoietic origin with the exception of platelets and red blood cells and their immediate precursors. CD45 is considered to be a pan leukocyte marker.

TECHNICAL SUMMARY.

Reactivity: The monoclonal antibody is directed against the CD45-antigen , defined T200 or Leucocyte Common Antigen. The antibody reacts with all cells of the haemopoietic lineage, not with cells of other lineages.

Specificity: The 180, 195, 205, 220, kD MW components of the leucocyte common antigen complex to be found on lymphocytes, monocytes, granulocytes, thymocytes and malignant T and B cells. Not reactivity has been observed with primary or metastatic carcinoma cells. Plasma cells or myeloma cells may have weak expression or be negative for this antigen.

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 TL1A 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 patiens with inmunodeficiency and autoinmune 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 immunosupressive activity, suggesting that CD45 may be a useful target for drug design

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PRINCIPLES OF THE TEST.

Immunostep CD45 OC515 monoclonal antibodies bind to the surface of cells that express the CD45 antigen. To identify these cells, peripheral blood leucocytes are incubated with the antibodies and red blood cells are lysed before washing to remove unbound antibodies. An appropriate fixative solution is added to lysed, washed cells before the stained and fixed cells are analysed by flow.

REAGENTS.

Cluster Designation:	CD45
WHO Classification:	Leukocyte Workshop IV.
Clone:	HI30
Isotype:	IgG1
Species:	Mouse
Composition:	IgG1 heavy chain Kappa light chain
Source:	Hybridome Cells
Immunogen:	Isolation of whole human peripheral blood mononuclear cells (PBMC's) and Tonsil cells
Method of Purification:	Affinity chromatography (Protein A/G)
Fluorochrome:	OC515. Excitation wavelength 405 nm and 407 nm Emission wavelength 515 nm
Suggested bandpass filter:	510/50 or 513/22 or 525/50 or 530/30 or 585/42
Dichroic mirror	502LP
Molar composition:	OC515/protein ± 3-5
Reagents contents:	0,5 ml vial containing monoclonal antibody for 100 tests, The conjugate is provided in aqueous buffered solution containing protein stabilizer, and ≤0.09% sodium Azide
Reagent preparation:	Ready to use.

1. STATEMENTS, SETTINGS AND WARNINGS.

- Reagents contain sodium azide. Sodium azide under acid conditions yields hydrazoic acid, an extremely toxic compound. Azide compounds should be diluted with running water before being discarded. These conditions are recommended to avoid deposits in plumbing where explosive conditions may develop.
- Light exposure should be avoided. Use dim light during handling, incubation with cells and prior to analysis.
- Do not pipet by mouth.
- Samples should be handled as if capable of transmitting infection. Appropriate disposal methods should be used.
- The sample preparation procedure employs a fixative (formaldehyde). Contact is to be avoided with skin or mucous membranes.
- Do not use antibodies beyond the stated expiration dates of the products.
- Deviations from the recommended procedure enclosed within this product insert may invalidate the results of testing.
- FOR *IN VITRO* DIAGNOSTIC USE
- For professional use only.

2. APPROPIATE STORAGE CONDITIONS.

- CD45 OC515 Keep in dark place at 2-8°C. DO NOT FREEZE.

**Note: it's been described stored conjugated monoclonal antibodies on OC515 at -20°C. This can affect to the conjugated intense.*

we know how
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3. EVIDENCE OF DETERIORATION.

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

- The normal appearance of the OC515 conjugated monoclonal antibody is a clear yellow-orange liquid.

4. SPECIMEN COLLECTION.

Collect venous blood samples into blood collection tubes using an appropriate anticoagulant (EDTA or heparin). For optimal results the sample should be processed within 6 hours of venipuncture. EDTA, ACD or heparin may be used if the blood sample is processed for analysis within 30 hours of venipuncture. ACD or heparin, but not EDTA, may be used if the sample is not processed within 30 hours of venipuncture. Samples that cannot be processed within 48 hours should be discarded.

If venous blood samples are collected into ACD for flow cytometric analysis, a separate venous blood sample should be collected into EDTA if a CBC is required.

Unstained anticoagulated blood should be retained at 20-25°C prior to sample processing. Blood samples that are hemolyzed, clotted or appear to be lipemic, discoloured or to contain interfering substances should be discarded.

Refer to "*Standard Procedures for the Collection of Diagnostic Blood Specimens*" published by the National Committee for Clinical Laboratory Standards (NCCLS) for additional information on the collection of blood specimens.

5. SAMPLE PREPARATION.

1. From a collect blood into an appropriate anticoagulan mixed with EDTA (until the process moment, keep in cold). Determine cell viability using 7ADD or Propidium Iodide. If the cell viability is not at least 85%, the blood sample should be discarded.
2. Pipette 100µl of well mixed blood into 12 x 75 mm polypropylene centrifuge tubes marked unknown and control.
3. Add 20µl of Immunostep CD45 OC515-conjugated monoclonal antibody and 180µl of phosphate buffered saline (PBS) to tubes marked unknown. In other control tube add 5 µl of corresponding Immunostep IgG1 OC515-conjugated isotypic control reagent. Mix gently.
4. Incubate all tubes for 15 minutes at room temperature (22 ± 3°C) in the dark.
5. Add lysing solution to all tubes according to the manufacturer's directions.
6. Centrifuge all tubes at 400 x g for 3 minutes at room temperature.
7. Add fixing solution to all tubes according to the manufacturer protocol. Retain cells in fixing solution for not less than 30 minutes at room temperature (22 ±3°C) in the dark.
8. Wash the cells in all tubes twice with 4mL of PBS. Centrifuge at 400 x g for 3 minutes after each wash procedure.
9. Resuspend the cells from the final wash in 1 ml of PBS and store tubes at 2-8°C in the dark until flow cytometric analysis is performed. It is recommended that analysis be performed within 24-48 hours of staining and fixation.
10. Analyze on a flow cytometer according to the manufacturer instructions. For alternate methods of whole blood lysis, refer to the manufacturer recommended procedure.

6. MATERIALS REQUIRED BUT NOT SUPPLIED.

Isotype control reagents: Mouse IgG1, kappa: OC515

Serofuge or equivalent centrifuge

12 x 75 mm polypropylene centrifuge tubes

Micropipette capable of dispensing 5 µl, 20 µl,
100 µl, and 500 µl volumes

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Blood collection tubes with anticoagulant

Phosphate buffered saline (PBS)

7ADD or Propidium Iodide, 0.25% (w/v) in PBS for the determination of cell viability

Lysing Solution

Fixing Solution

Flow cytometer: Becton Dickinson FACSCaliburTM, Coulter Profile or equivalent 405 nm violet-equipped and appropriate computer hardware and software

7. INTERPRETATION OF RESULTS.

a. *FLOW CYTOMETRY*

Analyze antibody-stained cells on an appropriate flow cytometer analyzer according to the manufacturer instructions. The right angle light scatter or other scatter (SSC) versus forward angle light scatter (FSC) is collected to reveal the lymphocyte cell cluster. A gate is drawn for the lymphocyte cluster (lymphocyte bitmap). The fluorescence attributable to the OC515- conjugated monoclonal antibody is collected, and the percentage of antibody-stained lymphocytes, monocytes, granulocytes, thymocytes and malignant T and B cells is determined. An appropriate OC515- conjugated isotypic control of the same heavy chain immunoglobulin class and antibody concentration must be used to estimate and correct for non-specific binding to lymphocytes. An analysis region is set to exclude background fluorescence and to include positively stained cells. The following histograms are representative of cells stained and region from a normal donor.

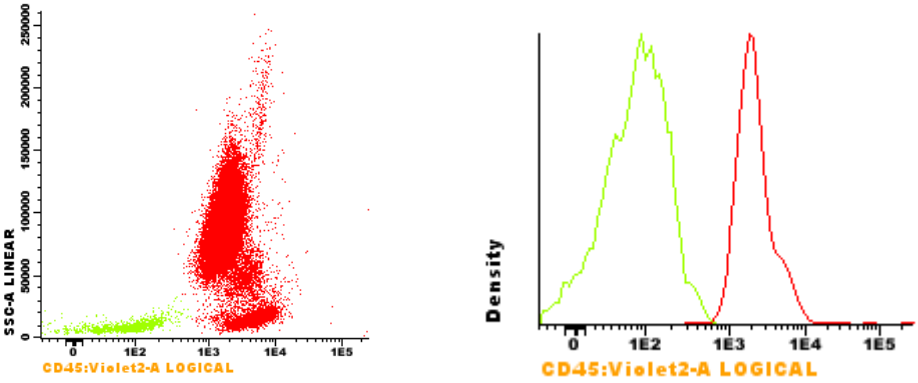

Fig. 1: CD45 OC515 vs Side Scatter transformed dot-plot

Cells were analyzed on a FACSAria (Becton Dickinson, San Jose, CA) flow cytometer, using BD FACSDiva software.

8. QUALITY CONTROL PROCEDURES.

Non-specific fluorescence identified by the FITC, OC515 and CFBlue conjugated isotypic control is usually less than 2% in normal individuals. Non-specific fluorescence identified by the PE and APC and their tandems conjugated isotypic controls are usually less than 4% in normal individuals. If the background level exceeds these values, test results may be in error. Increased non-specific fluorescence may be seen in some disease states.

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A blood sample from each normal and abnormal donor should be stained with the CD45 Pan-lymphocyte and CD14 Pan-monocyte monoclonal antibodies. When used in combination, these reagents assist in identifying the lymphocyte analysis region, and distinguish lymphocytes from monocytes, granulocytes and unlysed or nucleated red cells and cellular debris.

A blood sample from a healthy normal donor should be analyzed as a positive control on a daily basis or as frequently as needed to ensure proper laboratory working conditions. Each laboratory should establish their own normal ranges, since values obtained from normal samples may vary from laboratory to laboratory.

An appropriate isotype control should be used as a negative control with each patient sample to identify non-specific Fc binding to lymphocytes, monocytes and granulocytes. An analysis region should be set to exclude the non-specific fluorescence identified by the isotypic control, and to include the brighter fluorescence of the lymphocyte, monocytes and granulocytes population that is identified by the specific antibody.

Refer to the appropriate flow cytometer instrument manuals and other available references for recommended instrument calibration procedures.

9. LIMITATIONS OF THE PROCEDURE.

1. Incubation of antibody with cells for other than the recommended time and temperature may result in capping or loss of antigenic determinants from the cell surface.
2. The values obtained from normal individuals may vary from laboratory to laboratory; therefore, it is recommended that each laboratory establish its own normal range.
3. Abnormal cells or cell lines may have a higher antigen density than normal cells. This could, in some cases, require the use of a larger quantity of monoclonal antibody than is indicated in the procedures for Sample Preparation.
4. Blood samples from abnormal donors may not always show abnormal values for the percentage of lymphocytes stained with a given monoclonal antibody. Results obtained by flow cytometric analysis should be considered in combination with results from other diagnostic procedures.
5. When using the whole blood method, red blood cells found in some abnormal donors, as well as nucleated red cells found in normal and abnormal donors may be resistant to lysis by lysing solutions. Longer red cell lysis periods may be needed to avoid the inclusion of unlysed red cells in the lymphocyte gated region.
6. Blood samples should not be refrigerated or retained at ambient temperature for an extensive period (longer than 24-30 hours) prior to incubating with monoclonal antibodies.
7. Accurate results with flow cytometric procedures depend on correct alignment and calibration of the laser, as well as proper gate settings.
8. Due to an unacceptable variance among the different laboratory methods for determining absolute lymphocyte counts, an assessment of the accuracy of the method used is necessary.
9. Al results need to be interpreted in the context of clinical features, complete immunophenotype and cell morphology, taking due account of samples containing a mixture of normal and neoplastic cells.

10. REFERENCE VALUES.

The cellular elements of human Bone Marrow include lymphocytes, monocytes, granulocytes, red blood cells and platelets.

Nucleated cells Percentage in the Bone Marrow

Cell type	Percentage
Progranulocytes	56,7
Neutrophils	53,6
Myeloblasts	0,9
Promyeloblasts	3,3
Promyelocytes	12,7
Metamyelocytes	15,9
Eosinophils	3,1
Basophils	<0,1
Proerythrocyte	25,6

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Proerythrblasts	0,6
Basophil Erythroblast	1,4
Polycromatic Erythroblast	21,6
Ortocromatic Erythroblast	2
Megakaryocytes	<0,2
Lymphocytes	16,2
Plasma cells	2,3
Reticular cells	0,4

Normal human peripheral blood lymphocytes 20-47% (n=150% confidence interval)

Nucleated cells Percentage in Peripheral Blood of a Normal Patient

Cell type	Percentage	Number of event.
Red Blood Count		3,8 - 5,6 $X10^6$/µL
Platelets		150 - 450 $X10^3$/µL
White Blood Count (WBC)		4.3 - 10.0 $X10^3$/µL
Neutrophils	57 – 67 %	1,5 - 7.0 $X10^3$/µL
Lymphocytes*	25 – 33 %	1.0 - 4.8 $X10^3$/µL
T cell	56 – 82 % of lymphocytes	
T cell CD4+	60 % of T cells	
T cell CD8+	40 % of T cells	
Cell NK+	6 – 33 of lymphocytes	
B cell	7.7 – 22 of lymphocytes	
Monocytes	3 – 7 %	0.28 - 0.8 $X10^3$/µL
Eosinophils	1 – 3 %	0.05 – 0,25 $X10^3$/µL
Basophils	0 – 0,075 %	0,015 – 0,05 $X10^3$/µL
Reticulocyte	0,5 – 1,5 % of total Red Blood Cell	

Expected values for pediatrics and adolescents have not been established.
The values obtained from normal individuals may vary from laboratory to laboratory; therefore, it is recommended that each laboratory establish its own normal range.

11. PERFORMANCE CHARACTERISTICS.

a. *SPECIFICITY*

HI30 was assigned to CD45 during the IV HLDA Workshops on Human Leucocyte Differentiation Antigens (Code WS: 816)

Anti-CD45 OC515 recognizes human leucocyte antigens, the 180, 195, 205, 220, kD MW components of the leucocyte common antigen complex to be found on lymphocytes, monocytes, granulocytes, thymocytes and malignant T and B cells.

To analyse the specificity of CD45 OC515, we used blood samples which were obtained from healthy normal donors of Caucasian and were stained with Immunostep CD45 OC515, CD41 APC and CD235a PE 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 and ammonium chloride as lysis solution.

To evaluate the reagent's Specificity (cross-reactivity with other cell populations), 10 blood samples from healthy donors were studied, stained with the MAb to study. The percentage of leukocytes, platelets and Red bood cells stained with the mentioned MAb was evaluated. The results obtained are shown in the following table:

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Case Summaries[a]

		% Leukocytes	% Platelets	% Red bood cells
1		99,99	,12	,01
2		99,99	,00	,01
3		99,83	,14	,03
4		99,80	,17	,03
5		99,86	,10	,04
6		99,93	,04	,02
7		99,96	,01	,03
8		99,94	,01	,04
9		99,90	,02	,08
10		99,85	,07	,08
Total	N	10	10	10
	Mean	99,9050	,0680	,0370
	Median	99,9150	,0550	,0300
	Std. Deviation	,06754	,06125	,02497
	Minimum	99,80	,00	,01
	Maximum	99,99	,17	,08
	Range	,19	,17	,07

a. Limited to first 100 cases.

b. *SENSIBILITY*

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

Case Summaries[a]

	% Expected	% Obtained
1	95,28	95,28
2	83,37	87,21
3	71,46	84,12
4	59,55	77,02
5	47,64	64,52

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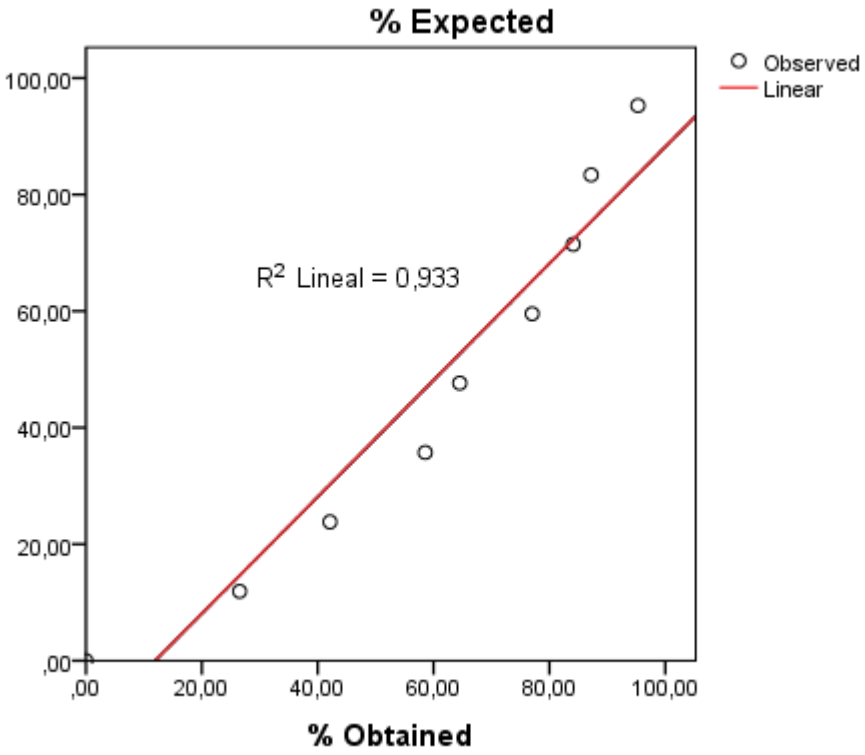
6		35,73	58,56
7		23,82	42,11
8		11,91	26,51
9		,00	,00
Total	N	9	9
	Mean	47,6400	59,4811
	Median	47,6400	64,5200
	Std. Deviation	32,61688	31,46075
	Variance	1063,861	989,779

a. Limited to first 100 cases.

Model Summary

Model	R	R Square	R Square Change	Adjusted R Square
1	,996(a)	,933	,933	,923

a Predictors: (Constant), Expected



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c. REPRODUCIBILITY

Reproducibility for the Immunostep CD45 OC515-conjugated monoclonal antibodies was determined by performing 10 replicated determinations of each three Leukocytes ranges; high, medium and low. Thus, a total of 10 determinations were performed for each group, reproducibility therefore was demonstrated throughout the entire measuring range.

The 10 determinations for each range were performed by analysis of 10 separate samples. Leukocytes 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, mid and low range percentage of Leukocytes.

Case Summaries[a]

	% High	% Medium	% Low
1	86,70	91,17	88,85
2	86,79	91,53	89,22
3	86,81	91,21	90,43
4	86,80	90,90	90,01
5	86,39	91,34	89,86
6	86,37	91,17	88,68
7	86,08	90,65	90,17
8	85,35	91,20	90,69
9	84,28	90,58	90,44
10	78,36	91,03	89,81
Total N	10	10	10

a. Limited to first 100 cases.

This table shows the percentage of positive cells for each acquisition

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
% High	10	78,36	86,81	85,3930	2,59854
% Medium	10	90,58	91,53	91,0780	,29578
% Low	10	88,68	90,69	89,8160	,68913
Valid N (listwise)	10				

**Note:* *Data analyzed with SPSS for Windows 11.0.1*

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d. REPEATABILITY OR PRECISION

To determine the precision or repeatability of this product, two samples were stained with two different lots. The following table shows the data on the mean fluorescence intensity, standard deviation and coefficient of variation between batches analysed for each of the samples.

Descriptive Statistics

	Mean	Median	Total %	Std. Deviation	CV
Lot 1 Sample 1	1031,15	939,99	99,68	459,05	44,52
Lot 2 Sample 1	1025,28	922,77	99,67	484,14	47,22
Lot 1 Sample 2	1082,90	968,55	99,29	538,00	49,68
Lot 2 Sample 2	1008,89	914,42	99,14	482,07	47,78

12. BIBLIOGRAPHY.

1. Krensky AM, Sanchez-Madrid F, Robbins E, Nagy JA, Springer TA, Burakoff SJ. The functional significance, distribution, and structure of LFA-1, LFA-2, and LFA-3: cell surface antigens associated with CTL-target interactions. J Immunol. 1983;131:611-616

2. Escribano L, Orfao A, Villarrubia J, et al. Immunophenotypic characterization of human bone marrow mast cells: a flow cytometric study of normal and pathologic bone marrow samples. Anal Cell Pathol. 1998;16:151-159

3. Schwinzer R. Cluster report: CD45/CD45R. In: Knapp W, Dörken B, Gilks WR, et al, eds. Leucocyte Typing IV:White Cell Differentiation Antigens. New York, NY: Oxford University Press; 1989:628-634.

4. Jackson A. Basic phenotyping of lymphocytes: selection and testing of reagents and interpretation of data.Clin Immunol Newslett. 1990;10:49-55.