Anti- Human CD8 (143-44)

Fluorochrome	Reference	Test
FITC	8F1-100T	100 test
PE	8PE1-100T	100 test
APC	8A1-100T	100 test

PRODUCT DESCRIPTION

Other Names: T8, Leu2, T-cell surface glycoprotein CD8 alpha chain, T-lymphocyte differentiation antigen T8/Leu-2 T8/Leu-2
Description: The anti-CD8 monoclonal antibody derives from the hybridisation of mouse SP2 myeloma cells and spleen cells from BALB/c mice immunised with human T lymphocytes. The antibody is formed by an IgG1 heavy chain and a kappa light chain.
Clone: 143-44
HLDA: The anti-CD8 antibody, clone 143-44, was included in the Fourth Workshop on Human Leukocyte Differentiation Antigens, using Code 169.
Isotype: Mouse IgG1, kappa
Reactivity: Human
Source: Supernatant proceeding from an *in vitro* cell culture of a cell hybridoma.
Purification: Affinity chromatography.
Composition: Mouse anti-human CD8 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)	20 ug in 2 ml	10
PE (R-Phycoerythrin)	10 ug in 2 ml	5
APC (Allophycocyanin)	20 ug in 2 ml	10

RECOMMENDED USAGE

Immunostep's CD8, clone 143-44, is a monoclonal antibody intended for *in vitro* diagnostic use in the identification and enumeration of human sample lymphocytes that express CD8 using flow cytometry.

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, including agammaglobulinemia[9], Severe Combined Immunodeficiency (SCID)[10] and the Acquired Immunodeficiency Syndrome (AIDS)[11].

An increase in the number of CD8+ lymphocytes has been acknowledged in infections with viruses such as cytomegalovirus, hepatitis B and HIV[1, 11].

PRINCIPLES OF THE TEST

The anti-CD8 monoclonal antibody binds to the surface of cells that express the CD8 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
FITC	Mouse IgG1	ICIGG1F-100UG
PE		ICIGG1PE-50UG
APC		ICIGG1A-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 CD8 and determine the percentage of stainend T 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 CD8, 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:

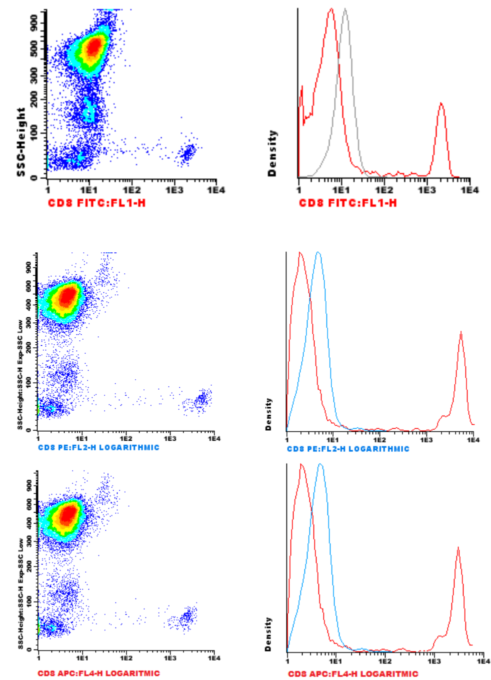

Fig. 1: On the left, a biparametric diagram of the average fluorescence intensity of the CD8+ T lymphocyte population and its internal complexity (SSC) in a peripheral blood specimen from a healthy donor. On the right, a diagram of the same specimen in histogram format.

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.
6. In order to obtain the best values, they should be 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[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-CD8 antibody, clone 143-44, was included in the Fourth Workshop on Human Leukocyte Differentiation Antigens (HDLA), using Code 169[7].

The antibody is directed against the CD8 antigen, also known as T8 antigen or Leu2, expressed in 20-30% of peripheral blood T lymphocytes, 60% of thymocytes and a limited number of diseases of T-cell origin. Normal B lymphocytes, monocytes, red blood cells, platelets or granulocytes do not express surface CD8 antigen[6].

In order to analyse specificity, 10 samples from healthy Caucasian donors were obtained. The samples were stained with CD8 FITC monoclonal antibodies and processed following the protocol described in point 6. Moreover, other specific antibodies from the populations analysed were used. CD8 positive cells were selected from the T lymphocytes, monocytes, neutrophils, B lymphocytes, red blood cells and platelets regions. Moreover, the percentage of positive cells in the CD8 FITC region was analysed for IgG1 isotype control conjugated with FITC.

The results obtained are shown in the following table:

Descriptive statistics

	Minimun	Maximum	Mean	Standard Deviation
% Isotype control	,00	,09	,0500	,03162
% T lymphocytes	3,71	9,47	6,50	2,49643
% Platelets	,00	,28	,0990	,09445
% Erythrocytes	,00	,09	,0310	,02470
% B lymphocytes	,01	,03	,0170	,00823
% Monocytes	,01	,05	,0210	,01595
% Neutrophils	,00	1,00	,1060	,31423
Valid N	10			

LINEARITY

For the linearity analysis, different dilutions of a positive population (CD 8 T lymphocytes) and a negative population (neutrophils) 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	Std. Error of the Estimate	Linear regression
1	,997	,993	Y= 1,012X - 0,479

REPEATABILITY AND PRECISION BETWEEN BATCHES

The repeatability of CD8, clone 143-44, monoclonal antibodies was established by performing 10 replicates of 10 anticoagulated peripheral blood specimens from healthy donors with different lymphocyte ranges. Moreover, the level of precision between batches was assessed using two or three different batches of antibodies for each sample[8].

This makes a total of 200-300 determinations for analysing the antibody repeatability and its precision between batches. The results obtained are shown in the following table:

		Repeability		Between-Lot Precission	
	Parameter	Standard Deviation	% CV	Standard Deviation	% CV
FITC	Mean Fluorescence Intensity	178,27	2,51	412,19	5,53
	% Positive cells	0,34	4,51	0,61	7,6
PE	Mean Fluorescence Intensity	1982,75	10,73	1864,03	10,71
	% Positive cells	0,22	2,45	0,07	0,76
APC	Mean Fluorescence Intensity	5513,01	10,53	9,81	
	% Positive cells	0,22	1,90	0,01	0,11

REPRODUCIBILITY

In order to demonstrate reproducibility or inter-laboratory precision, five replicates of five different anticoagulated peripheral blood samples from healthy donors were stained and stabilized using cell stabiliser. The samples were acquired during a period of five days in three separate laboratories.

A total of 375 determinations were made to show the inter-laboratory precision of CD8, clone 143-44.

The test results are shown in the following grid:

	Between-Days Precission		Between-Lab 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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