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Produktinformation

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- Diagnostik & molekulare Diagnostik
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Anti- Human CD7 (HIT7)

Fluorochrome	Reference	Test
FITC	7F-100T	100 test
APC	7A-100T	100 test

IVD CE immunostep we know how

PRODUCT DESCRIPTION

Other Names: T cells antigen, CD7, GP-40, T cells leukemia antigen, cell surface antigen Leu-9, TP41.
Description: The anti-CD7 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: HIT7
HLDA: The anti-CD7 antibody, clone HIT7, was included in the 5th Workshop on Human Leukocyte Differentiation Antigens, using Code T-CD07.03 .
Isotype: Mouse IgG1, kappa
Reactivity: Human
Source: Ascitic fluid from mouse immunized with hybridoma producing cells.
Purification: Affinity chromatography.
Compositión: Mouse anti-human CD7 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 isotiocyanate)	50 ug en 2 ml	25
APC (Allophycocyanin)	25 ug en 2 ml	12,5

RECOMMENDED USAGE

Immunostep's CD7, clone HIT7, is a monoclonal antibody intended for *in vitro* diagnostic use in the identification and enumeration of human sample lymphocytes that express CD7 using flow cytometry.

CLINICAL RELEVANCE

This antibody can be used in flow cytometry for analysis of blood samples and bone marrow or immunohistochemistry. The CD7 antigen is also expressible in myeloblastic leukemias. It is suitable for the identification of T cell neoplasias by immunohistochemistry. The CD7 is also used to analyze subsets of T and NK cells and to characterize all T cells and other lymphoid leukaemias of T-cells.

PRINCIPLES OF THE TEST

The anti-CD7 monoclonal antibody binds to the surface of cells that express the CD7 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)[11,12]. 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
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 CD7 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 CD7, 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 applying the protocol described in point 6:

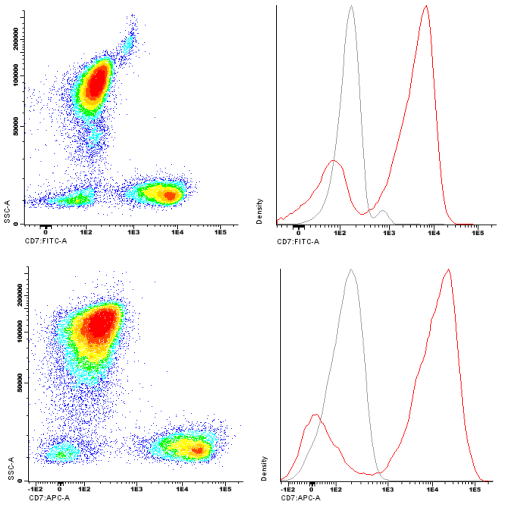

Fig. 1: On the left, a biparametric diagram of the average fluorescence intensity of the CD7+ lymphocyte population and its internal complexity (SSC) in a peripheral blood specimen from a healthy donor. On the right, a diagram of the same specimen in histogram format.

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. 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-CD7 clone HIT7 was included in the 5th workshop on differentiation antigens of human leukocytes (HLDA) with the code T-CD07.03

This antibody is directed against the CD7 antigen also called antigen GP40, Leu9, expressed during differentiation of T lymphocytes and in the 85% -90% of peripheral blood T lymphocytes. Under normal conditions recognizes the total of CD8 + T cells and approximately 90% of CD4 + T cells as well as most of NK cells. The CD7 is negative or very weak in granulocytes, monocytes, B, platelets and erythrocytes[7].

To analyze the specificity 10 samples obtained from healthy caucasian donors were evaluated. Samples were stained with CD7 FITC monoclonal antibody and processed according to the protocol described in paragraph 6. In addition, specific antibodies of different populations analyzed were used.

CD7 positive cells of lymphocytes, monocytes, and neutrophils region were selected.
The result is shown in the following table:

Descriptive statistics

	Media	Median	Typical desviation
% Lymphocytes	59,3850	60,2300	5,61264
% Monocytes	18,5190	18,3150	7,03061
% Neutrophils	17,8120	17,2200	6,07972
Valids N	10	10	10

LINEARITY

For linearity analysis, different dilutions of a normal sample of peripheral blood, were performed maintaining total number of cells constant and analyzing the expected percentages with the percentages obtained.

Data obtained for the CD7 APC are shown in the following table:

R	R square	Standard error of estimate	Linear regression
1	,995	,44439	Y= 0,957X - 0,546

Repeatability and accuracy BETWEEN LOTS

The repeatability of CD7 monoclonal antibody clone HIT7 was determined performing 10 replicates of anticoagulated peripheral blood from 10 healthy individuals with different ranges of lymphocytes. Moreover accuracy between batches was analyzed using three different lots of antibody CD7 APC for each sample[8].

This makes a total of 300 tests to analyze antibody repeatability and accuracy between batches. CD7 FITC antibody precision between lots was analyzed using two different lots of antibody. This makes a total of 200 tests to analyze the repeatability and accuracy between batches. The results are shown in the following table:

		repeatability		Accuracy between lots	
	Analysed parameter	Typical desviation	% CV	Typical desviation	% CV
FITC	IMF	64,81	2,55	35,34	1,45
	% positive cells	0,96	4,34	0,46	2,09
APC	IMF	3196,51	22,22	3033,96	20,90
	% positive cells	2,28	9,10	1,05	4,02

Reproducibility

To demonstrate reproducibility inter-laboratory or precision, 5 replicates of 5 different anticoagulated peripheral blood samples from healthy donors were stained and stabilized with Cellular stabilizer[8]. Samples were acquired for 5 days in three different laboratories.

A total of 375 measurements were performed to demonstrate the inter-laboratory precision of CD7 HIT7 clone.

The test result is shown in the following table:

	Precision between days		Precision inter-laboratories	
Parameter	SD	% CV	SD	% CV
% positive cells	2,00	11,30	2,97	16,76

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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