

# Anti-Human CD9 (VJ1/20)

Fluorochrome	Reference	Test
FITC	9F-100T	100 test
APC	9A-100T	100 test



## PRODUCT DESCRIPTION

**Other Names:** DRAP-27, MRP-1, p24, CD9 antigen, 5H9 antigen, Cell growth-inhibiting gene 2 protein, Leukocyte antigen MIC3, Motility-related protein, MRP-1, Tetraspanin-29, Tspan-31

**Description:** The anti-CD9 monoclonal antibody derives from tissue / cell preparation (Human tonsil).

**Clone:** VJ1/20

**Isotype:** Mouse IgG2a, kappa

**Reactivity:** Human

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

**Purification:** Affinity chromatography.

**Composition:** Mouse anti-human CD9 monoclonal antibody conjugated with a fluorochrome and in an aqueous solution which contains stabilising protein and 0.09% sodium azide (NaN<sub>3</sub>).

Fluorochrome	Reagent provided	Concentration (µg/ml)
FITC (Fluorescein isothiocyanate)	50 ug in 2 ml	25
APC (Allophycocyanin)	25 ug in 2 ml	12,5

## RECOMMENDED USAGE

Immunostep's CD9, clone VJ1/20, is a monoclonal antibody intended for *in vitro* diagnostic use in the identification and enumeration of human sample platelets that express CD9 using flow cytometry.

## CLINICAL RELEVANCE

CD9 monoclonal antibody inhibits infection by FIV and CDV (canine distemper virus) but CD9 does not directly bind the virus, suggesting a role as a viral co-receptor, as for diphtheria toxin.

CD9 monoclonal antibody has been used for immunophenotyping leukemias and bone marrow purging in autologous bone marrow transplantation. The expression of CD9 is inversely correlated with metastasis in melanoma and breast cancer. CD9 expression is inversely correlated with actuarial survival in lung cancer.

Transfection of CD9 in murine melanoma cells has been shown to reduce metastasis<sup>1-3</sup>

## PRINCIPLES OF THE TEST

The anti-CD9 monoclonal antibody binds to the surface of cells that express the CD9 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](mailto: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](http://www.immunostep.com)
- Avoid microbial contamination of the reagent.
- Protect from light. Use dim light during handling, incubation with cells and prior to analysis.
- Never mouth pipette.
- In the case of contact with skin, wash in plenty of water.
- The samples should be handled in the same way as those capable of transmitting infection. Appropriate handling procedures should be guaranteed.
- Do not use after the expiry date indicated on the vial.
- Deviations from the recommended procedure could invalidate the analysis results.
- FOR *IN VITRO* DIAGNOSTIC USE.
- For professional use only.
- 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)<sup>3,4,5</sup>. 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 IgG2a	ICIGG2AF-100
APC		ICIGG2AA-50

- 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. Centrifuge tube of freshly drawn EDTA blood (600rpm or 75xg, 20')
2. Remove platelets (top layer) and wash twice over in PBS and BSA 2% and resuspend in PBS.
3. Add the appropriate volume of the antibody and mix gently with a vortex mixer. The optimal volume should be determined by the individual laboratory.
4. Incubate in the dark at room temperature (20-25 °C) for 15 minutes.
5. The recommended negative control is a non-reactive conjugated antibody of the same isotype (*please see materials required but not provided*).
6. Add 2 mL 0.01 mol/L PBS (It betters that it containing 2% bovine serum albumin) and resuspend the cells by using a vortex mixer.
7. Centrifuge at 1000 x g for 5 minutes. Gently aspirate the supernatant and discard it leaving approximately 50 µL of fluid.

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 CD9 and determine the percentage of stained cells.

It is necessary to use an isotype control conjugated with the same fluorochrome, of the same type of immunoglobulin heavy chain and concentration as that of the CD9, so as to evaluate and correct the unspecific binding of leucocytes (*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 cells:

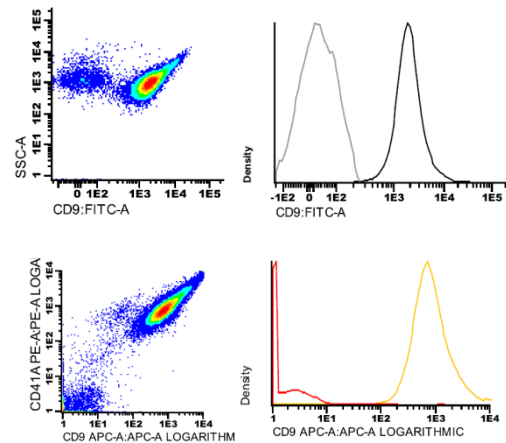


Fig. 1: On the left, a biparametric diagram of the average fluorescence intensity of platelets stained with CD9+ and its internal complexity (SSC). Right, a diagram of the same specimen in histogram format.

## 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<sup>6,7,8</sup>

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 antigen is expressed on eosinophils, granulocytes, monocytes and numerous stromal cells.

This antibody reacts immunohistochemically on frozen sections of platelets, monocytes and pre-B cells with differential staining on lymphoid and epithelial tissues.

To evaluate the reagent's Specificity (cross-reactivity with other cell populations), 10 blood samples from healthy donors were studied, stained with an adequate isotype control and the Monoclonal antibody to study. The percentage of platelets and erythrocytes stained with the mentioned Monoclonal antibody was evaluated.

The results obtained are shown in the following table:

			Platelets	Erythrocytes
	APC	N	Valid	10
		Missing	0	0
Mean		97,6780	26,5090	
Median		98,4950	24,8450	
Mode		92,93 (a)	,00 (a)	
Std. Deviation		2,18925	19,34248	
Variance		4,79280	374,13157	
Range		6,61	51,39	
FITC				Platelets
	N	Valid	10	10
		Missing	0	0
	Mean		96,9820	5,6750
	Median		97,7500	4,0950
	Mode		92,36 (a)	1,19 (a)
	Std. Deviation		1,86282	4,36708
	Variance		3,47008	19,07138
	Range		6,64	14,46

### SENSIBILITY

Sensitivity of the Immunostep CD9 monoclonal antibodies was determined by staining a blood sample from normal 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 Square	Std. Error of the Estimate
APC	0,999 <sup>(a)</sup>	0,998	0,998	1,30586
FITC	0,984 <sup>(a)</sup>	0,969	0,964	5,32595

(a) Predictors: (Constant), Obtained

## REPRODUCIBILITY

Reproducibility for the Immunostep CD9 monoclonal antibodies was determined by performing 10 replicated determinations of each antibody in each of three CD9+ ranges, high, medium and low. Thus, a total of 30 determinations were performed for each form of CD9. 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. Platelets 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 CD9+ cells. Mid-range and low range samples were obtained by mixing known CD9- 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.

		N	Minimum	Maximum	Mean	Std. Deviation
APC	High	10	97,70	98,82	98,34	0,37
	Medium	10	97,15	98,02	97,72	0,25
	Low	10	95,88	97,14	96,45	0,37
	Valid N (listwise)	10				
FITC	High	10	71,86	77,75	74,06	0,63
	Medium	10	63,34	69,41	67,74	0,29
	Low	10	61,28	63,63	62,5	0,10
	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.

## REFERENCES

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7. Kotylo PK et al. Reference ranges for lymphocyte subsets in pediatric patients. Am J Clin Pathol 100:111-5 (1993)
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**SURVEILLANCE AND NOTIFICATION** 

In accordance with Annex I, Section 20.4.1(n) of Regulation (EU) 2017/746, the user is obliged to report any serious incident related to the use of the product.

- **To the Manufacturer:** Please contact our Surveillance Department at [vigilancia@immunostep.com](mailto:vigilancia@immunostep.com)
- **To the Competent Authority:** Report via the official channels of the Member State.

**EXPLANATION OF SYMBOLS**

	CE labeling
	<i>In vitro</i> diagnostic device
	Manufacturer
	Pay attention to