

# Anti- Human CD300e (IREM2) (UP-H2)

Fluorochrome	Reference	Test
APC	IREM2A-100T	100 test



## PRODUCT DESCRIPTION

**Other Names:** alfa-Mar; CD300e; CLM-2; CMRF35-A5

**Description:** The anti-IREM2 monoclonal antibody derives from 300.19 cells transfected with HA-tagged IREM-2. The antibody is formed by an IgG1 heavy chain and a kappa light chain.

**Clone:** UP-H2

**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 IREM2 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)
APC (Allophycocyanin)	25 ug in 2 ml	12,5

## INTENDED USE

Anti-IREM2 APC is a fluorochrome-conjugated antibody used for the identification of cell populations presents in human biological samples that express IREM-2 antigen on their membrane by flow cytometry.

## CLINICAL RELEVANCE

This reagent can be used for immunophenotyping by flow cytometry.

Anti-IREM2 is an antibody specific for monocytes and myeloid dendritic cell line. In normal peripheral blood should be positive for 80% of monocytes.

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 relationship between the normal expression of this antigen and the expression of other relevant antigens in order to perform an appropriate analysis and the correct interpretation of results<sup>5,6</sup>.

Monoclonal antibody anti-IREM2 of Immunostep may be used as aid to diagnostic in the characterization of classifying different types of myeloid leukemias, especially monocytic line<sup>2</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.

## PRINCIPLES OF THE TEST

The anti-IREM-2 monoclonal antibody reagent enables the identification of cells that express the IREM-2 surface antigen.

Flow cytometry assays, use fluorochrome-conjugated antibodies binding to specific antigens expressed by the target cells.

Analysis of the sample is based on the detection of characteristic light emissions emitted by the fluorescently labelled antibody upon excitation with laser lights. The collected data can be processed and analysed using flow cytometry software.

## 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 or false results may occur.
- 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.
- All biological samples must be handled and treated as biohazards. Appropriate disposing procedures for infectious material have to be guaranteed according European Regulations.
- Stability at +2 °C to +8 °C storage. Refer to the use-by date indicated on the vial label. Do not use after the expiry date indicated on the vial.

- h) Deviations from the recommended procedure could invalidate the analysis results.
- i) Analysis results obtained with the reagent cannot be used for classification of disease states in exclusive.
- j) Interpretation of results is under responsibility of the end user.
- k) FOR *IN VITRO* DIAGNOSTIC USE.
- l) For professional use only.
- m) Before acquiring the samples, it is necessary to make sure that the flow cytometer is calibrated.
- n) When using an antibody multiple labelled panels, the different fluorochromes emit at different wavelengths but have some spectral overlap that must be corrected by cytometer compensation.

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.

**MATERIALS REQUIRED BUT NOT PROVIDED**

- Isotype controls:

Fluorochrome	Isotype control	Immunostep Reference
APC	Mouse IgG1	ICIGGIA-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 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</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. Samples with large number of nonviable cells can give erroneous results.

Viability of samples should be assessed. Samples with at least 80% viable cells is suggested in order to minimize risk of erroneous results. Human samples must be collected following European and national legislation.

**PROCEDURE:**

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<sup>6</sup> cells) and mix properly in the vortex.

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 hours after lysis.

**FLOW CYTOMETRY ANALYSIS**

Collect the fluorescence attributed to monoclonal antibody IREM2 and determine the percentage of stained monocytic 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 IREM2, 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:

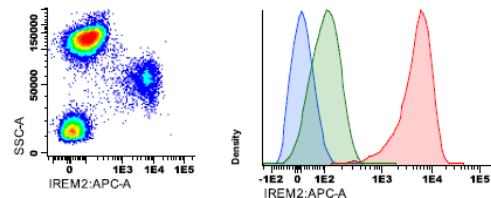


Fig. 1: On the left, a biparametric diagram of the average fluorescence intensity of the IREM2+ monocytes population and its internal complexity (SSC) in a peripheral blood specimen from a healthy donor. On the right, a diagram of the same specimen in histogram format.

**QUALITY CONTROL**

It is recommended running a blood control sample from a normal specimen or commercially available whole blood control to optimize instrument settings and as a quality control check point.

**PERFORMANCE CHARACTERISTICS**

**Precision**

**Especificidad analítica**

**Analytical specificity**

Analytical specificity was evaluated by comparing clone UP-H2 to a relevant reference clone of the same specificity. Reactivity towards the same antigen was inferred from the antibody blocking capacity or the staining diagonal observed

during co-incubation of UP-H2 with the reference clone. Measurements were performed using different donor samples. All measurements were within the acceptance criterion.

**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. Single color immunofluorescence provides only limited information and is not the method for analysis of disease states. Multicolor immunophenotyping allows more precise definition of atypical cell populations and is highly recommended.
4. 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.
5. 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.
6. 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.
7. Reagent data was collected typically with EDTA-treated blood and can be affected by the use of other anticoagulants.
8. Accurate results with flow cytometric procedures depend on correct alignment and calibration of the lasers, as well as correct gate settings.

**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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5. Kotylo PK et al. Reference ranges for lymphocyte subsets in pediatric patients. Am J Clin Pathol 100:111-5 (1993)
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**EXPLANATION OF SYMBOLS**

	In vitro diagnostic		Caution
	Batch code		Temperature limit
	Use by		Keep dry
	Catalog number		CE Mark
	Consult instruction for use		This side up
	Manufactured by		Handle with care
	Quantity per test		Contains sufficient for <n> test
	Keep away from sunlight		Do not reuse

**MANUFACTURED BY**



**Immunostep S.L**

Avda. Universidad de Coimbra, s/n

Cancer Research Center (CIC)

Campus Miguel de Unamuno

37007 Salamanca (Spain)

Tel. (+34) 923 294 827

[www.immunostep.com](http://www.immunostep.com)