

Product: CD45RA PURIFIED

Cat. Ref: 45RAPU2-0,1 MG

Reagent provided: 0,1 MG (1mg/ml)

Description: Mouse monoclonal Anti-Human CD45RA is recommended for use in flow cytometry. This product allows simultaneous detection and enumeration of the helper/inducer T-cell subset and CD45RA+ cells. The antibody is provided in liquid form in buffer containing 1% bovine serum albumin (BSA) (Lote: 113K1364 / SIGMA) and 0,09% NaN₃, pH 7.2.

HLDA: 5th International Workshop on Human Leukocyte Differentiation Antigens WS Code T-CD45.30

Clone: GRT22

Isotypes: IgG1

Fluorochromes: PURIFIED (pass through 0,22 µm filter)

Reactivity: Antibody GRT22 recognizes all CD45 molecules containing the A region exon. Human CD45RA is expressed on all cells of hematopoietic origin, except erythrocytes. CD45RA is a transmembrane tyrosine phosphate which can exist in at least nine different isoforms resulting from tissue-specific alternative RNA splicing of exons 4-7 of a single gene coding for the various N-terminal peptide segments. The CD45RA isoform predominates on naive/resting T cells and medullary thymocytes.

Specificity: CD45RA, 220 kDa MW component of the Leucocyte Common Antigen complex located on some CD4+ T lymphocytes, B lymphocytes and CD8+ lymphocytes. The CD45RA+/CD4+ lymphocytes functionally exert suppressor/inducer activity in in vitro assay systems.

Storage: Store in the dark at 2-8 °C. Do not use after expiration date stamped on vial. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the product is suspected, contact our Technical Services. (tech@immunostep.com).

Application: It is recommended for use in flow cytometry. This reagent is effective for direct immunofluorescence staining of human tissue for flow cytometric analysis using 20 µl/10⁶ cells.

Precautions:

1. The device is not intended for clinical use including diagnosis, prognosis, and monitoring of a disease state, and it must not be used in conjunction with patient records or treatment.
2. This product contains sodium azide (NaN₃), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.
3. As with any product derived from biological sources, proper handling procedures should be used.

Flow Cytometry Protocol:

1. The starting material is peripheral blood (PB) or another sample of cells in suspension. This is permanently anticoagulated with EDTA (until the time of processing it should be stored at 4°C). If processing is to be carried out within a few hours, it may be more appropriate not to subject the cells to changes in temperature and leave the samples at room temperature.
2. Before starting the technique, a small sample is taken and read on a haematological counter to obtain the haemogram and determine the number of leukocytes.
3. Generally, 100 µL of PB are taken when the number of leukocytes is 10 x 10³ cells/µL and 200/ µL when the number of leukocytes is equal to 5 x 10³ cells/ µL.
4. 1 µg of the McAb is pipetted into each tube and the tubes are incubated for 15 min at room temperature in the darkness.
5. A washing is made with centrifugation at 2000 rpm for 5 min with 5 mL of PBS in order to remove the McAb not bound to its antigen.
6. Add 10 µL of mouse anti-IgG antibody conjugated with some fluorochrome (Ref. Code No. GOATPOLYFITCANTIMOUIGG2a) is added and the mixture is incubated at room temperature for 15 min in the darkness. The absence of light is necessary so that the fluorochrome will not deteriorate since it shows a high degree of photostability.
7. After the incubation period, an erythrocyte-lysing solution is added at the amount recommended by the manufacturer and the mixture is incubated at room temperature in the darkness (the blood should be well mixed with the lysing solution).

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8. The tubes are centrifuged at 2000 rpm for 5 minutes. The supernatant is removed with a Pasteur pipette or with a vacuum pump.
9. The cell pellet is resuspended and a final wash is made with 3-5 mL of PBS at 2000 rpm for 5 min.
10. After removing the supernatant and resuspending the cell pellet, some 300 µL of PBS is added and the readings on the flow cytometer are recorded.
11. Analyse on a flow cytometer or store at 2-8 °C in the dark until analysis. Samples can be run up to 24 hours after lysis.

FOR MORE INFORMATION, PLEASE VISIT OUR WEBSITE: www.citometriadeflujo.info

References:

1. Lima M, Almeida J, dos Anjos Teixeira M, Queiros ML, Justica B, Orfao A. The "ex vivo" patterns of CD2/CD7, CD57/CD11c, CD38/CD11b, CD45RA/CD45RO and CD11a/HLADR expression identify acute/early and chronic/late NK-cell activation states. Blood Cells Mol Dis. 2002 Mar-Apr; 28 (2): 181-90.
2. Davey FR, Gatter KC, Ralfkiaer E, Pulford KAF, Krissansen GW, Mason DY. Immunophenotyping of non-Hodgkin's lymphomas using a panel of antibodies on paraffin- embedded tissues. Am J Pathol 1987; 129: 54- 63.
3. Schmidt RE. Non- lineage/ natural killer section report: new and previously defined clusters. In: Knapp W, Dörken B, Gilks WR, Rieber EP, Schmidt RE, Stein H, et al., editors. Leucocyte typing IV. White cell differentiation antigens. Proceedings of the 4th International Workshop and Conference; 1989 Feb 21- 25; Vienna, Austria. Oxford, New York, Tokyo: Oxford University Press; 1989. p. 517- 42.

***Note: For research use only. Not for use in diagnostic procedures.**