

Product: CD45RA APC

Cat. Ref: 45RAA2-100T

Reagent provided: 100 test (20µl / test)

Description: Mouse monoclonal Anti-Human CD45RA APC, 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 conjugate 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

Clones: GRT22

Isotypes: IgG1

Fluorochromes: *Allophycocyanin (Febico, Far East Bio-Tech Co.)*

Reactivity: Antibody RP1/11.1 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 kD 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.

Preparation:

1. Transfer 100 µL of anticoagulated (EDTA) blood to a 12 x 75 mm polystyrene test tube (10⁶ cells).
2. Add 20 µL of CD45RA APC and mix gently with a vortex mixer. The 20 µL is a guideline only; the optimal volume should be determined by the individual laboratory.
3. The recommended negative control is a non-reactive APC-conjugated antibody of the same isotype (Code No. **ISCONTAPCIGG1**)
4. Incubate in the dark at room temperature at 4 °C for 15 minutes or at room temperature (20-25 °C) for 15 minutes.
5. Add 1,5 ml of Lysing Solution to each sample and mix gently with a vortex mixer. Incubate for 10 minutes at room temperature in the dark.
6. Centrifuge at 1000 x g for 5 minutes. Gently aspirate the supernatant and discard it leaving approximately 50 µL of fluid.
7. Add 2 mL 0.01 mol/l PBS (It better that it containing 2% bovine serum albumin) and resuspend the cells by using a vortex mixer.
8. Centrifuge at 1000 x g for 5 minutes. Gently aspirate the supernatant and discard it leaving approximately 50 µL of fluid.
9. Resuspend pellet in an appropriate fluid for flow cytometry, e.g. 0.5 ml PBS. The PBS should contain 1% paraformaldehyde (fixative) if samples are not analysed the same day.
10. 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.**