

Product: CD45RB APC

Cat. Ref: 45RBA-100T

Reagent provided: 100 test (20µl / test)

Description: Mouse monoclonal Anti-Human CD45RB APC is recommended for use in flow cytometry. 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.

Clone: MC5/2

Isotypes: IgG1

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

Reactivity: The CD45 molecule is also known as the Leukocyte Common Antigen (LCA) or T200 antigen, and is comprised of different glycoproteins ranging from 180-240 kD [1,2]. Expression of CD45 is found on all hemopoietic cells, e.g. granulocytes, monocytes, macrophages and lymphocytes, except mature erythroid cells. Detection of the different isoforms can distinguish, for example, between naive T cells and memory T cells, which is of interest in patients with immunodeficiency and autoimmune diseases.

Variations in CD45RB expression can discriminate between Th1 and Th2 cells, i.e. CD45RB-bright and CD45RB-dim respective.

Reacts with the 190, 205 and 220 kD isoforms of the cell-surface antigen CD45RB. CD45RB bright expression on T cells correlates with higher proliferation and IFN-g production in comparison to CD45RB dim expression. 90% of lymphocytes are CD45RB positive.

Specificity: The antibody reacts with the LCA complex expressed on all haemopoietic cells but not platelets (190, 205, 200 kDa).

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 CD45RB 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. **ISOCONTAPCIGG1**)
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.

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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. Bottomly, K., M. Luqman, L. Greenbaum, S. Carding, J. West, T. Pasqualini, and D.B. Murphy. 1989. A monoclonal antibody to murine CD45R distinguishes CD4 T cell populations that produce different cytokines. *Eur. J. Immunol.* 19: 617 - 623.
2. Johnson, P., L. Greenbaum, K. Bottomly, and I.S. Trowbridge. 1989. Identification of the alternatively spliced exons of murine CD45 (T200) required for reactivity with B220 and other T200-restricted antibodies. *J. Exp. Med.* 169: 1179 - 1184.
3. Dianzani, U., M. Luqman, J. Rojo, J. Yagi, J.L. Baron, A. Woods, C.A. Janeway, Jr., and K. Bottomly. 1990. Molecular associations on the T cell surface correlate with immunological memory. *Eur. J. Immunol.* 20: 2249 - 2257.
4. Hathcock, K.S., G. Laszlo, H.B. Dickler, S.O. Sharrow, P. Johnson, I.S. Trowbridge, and R.J. Hodes. 1992. Expression of variable.

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