

**Product:** CD45RB PURIFIED

**Cat. Ref:** 45RBPU-0,1MG

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

**Description:** Mouse monoclonal Anti-Human CD45RB PURIFIED is recommended for use in flow cytometry. The purified is provided in liquid form in buffer containing 1% bovine serum albumin (BSA) (Lote: 113K1364 / SIGMA) and 0,09% NaN<sub>3</sub>, pH 7.2.

**Clone:** MC5/2

**Isotypes:** IgG1

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

**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](mailto: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/10<sup>6</sup> 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 (NaN<sub>3</sub>), 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. 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 10<sup>3</sup> cells/µL and 200/ µL when the number of leukocytes is equal to 5 x 10<sup>3</sup> 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

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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).
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  $\mu$ L 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](http://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.**