

Product: CD45RC PE
Cat. Ref: 45RCPE-100T
Reagent provided: 100 test (20µl / test)
Description: Mouse monoclonal Anti-Human CD45RC PE, is recommended for use in flow cytometry. The conjugate is provided in liquid form in buffer containing Stabilizing Solution, PBS pH 7,4 ± 0,2
Clone: RP1/12
Isotypes: IgG1
Fluorochromes: *R-Phycoerythrin (Europa Bioproducts, Ely, Cambridge)*

Reactivity & Specificity: This antibody reacts with CD45RC on pre-B lymphocytes, B cells, CD8+ T suppressor/cytotoxic cells, and a subset of CD4+ T helper (Th) lymphocytes. It weakly reacts with thymocytes. CD45RC is a high-molecular-weight isoform of CD45 (Leukocyte Common Antigen); its level of expression distinguishes subpopulations of CD4+ T cells with Th1-like and Th2-like effector functions. Levels of expression of CD45RC have also been reported to distinguish resting from activated T cells at various stages of maturation. CD45 is a member of the Protein Tyrosine Phosphatase (PTP) family; its intracellular (COOH-terminal) region contains two PTP catalytic domains, and the extracellular region is highly variable due to alternative splicing of exons 4, 5, and 6 (designated A, B, and C, respectively), plus differing levels of glycosylation. The CD45 isoforms detected in the rat are cell type-, maturation-, and activation state-specific. The CD45 isoforms play complex roles in T-cell and B-cell antigen receptor signal transduction.

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 CD45RC PE 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 PE-conjugated antibody of the same isotype (Code No. **ISOCNTPEIGG1**)
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. Spickett, G.P., M.R. Brandon, D.W. Mason, A.F. Williams, and G.R. Woollett. 1983. MRC OX-22, a monoclonal antibody that labels a new subset of T lymphocytes and reacts with the high molecular weight form of the leukocyte-common antigen. *Exp. Med.* 158:795 – 810.
2. Woollett, G.R., A.N. Barclay, M. Puklavec, and A.F. Williams. 1985. Molecular and antigenic heterogeneity of the rat leukocyte-common antigen from thymocytes and T and B lymphocytes. *Eur. J. Immunol.* 15:168 – 173.
3. Mason, D.W., R.P. Arthur, M.J. Dallman, J.R. Green, G.P. Spickett, and M.L. Thomas. 1983. Functions of rat T-lymphocyte subsets isolated by means of monoclonal antibodies. *Immunol. Rev.* 74:57 – 82.
4. Arthur, R.P., and D. Mason. 1986. T cells that help B cell responses to soluble antigen are distinguishable from those producing interleukin 2 on mitogenic or allogeneic stimulation. *J. Exp. Med.* 163:774 – 786.
5. Fowell, D., A.J. McKnight, F. Powrie, R. Dyke, and D. Mason. 1991. Subsets of CD4 + T cells and their roles in the induction and prevention of autoimmunity. *Immunol. Rev.* 123:37 – 64.
6. Papp, I., K.J. Wieder, T. Sablinski, P.J. O'Connell, E.L. Milford, T.B. Strom, and J.W. Kupiec-Weglinski. 1992. Evidence for functional heterogeneity of rat CD4 + T cells in vivo. Differential expression of IL-2 and IL-4 mRNA in recipients of cardiac allografts. *J. Immunol.* 148:1308 – 1314.
7. Groen, H., F.A. Klatter, A.S. van Petersen, J.M. Pater, P. Nieuwenhuis, and J. Kampinga. 1993. Composition of rat CD4 + resting memory T-cell pool is influenced by major histocompatibility complex. *Transplant. Proc.* 25:2782 – 2783.

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