

Product: CD45 PECy5

Cat. Ref: 45PC2-100T

Reagent provided: 10 test (20µl/test)

Description: Monoclonal Mouse Anti-Human CD45 PECy5 is recommended for use in flow cytometry for identification and analysis of CD45⁺ 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.

Clone: IMMU19.2

Isotype: IgG1

Fluorochrome: R-Phycoerythrin (*Europa Bioproducts, Ely, Cambridge*) and Cyanin-5 (*Amersham Pharmacia*)

CD45 → 6th International Workshops on Human Leucocyte Differentiation, WS Code N-L 103

Reactivity: The CD45 monoclonal antibody is directed against the CD45- antigen, defined T200 or Leucocyte Common Antigen. The antibody reacts with all cells of the haemopoietic lineage, not with cells of other lineages.

Specificity: The CD45 antibody recognizes 180, 195, 205, 220, kD MW components of the leucocyte common antigen complex to be found on lymphocytes, monocytes, granulocytes, thymocytes and malignant T and B cells. No reactivity has been observed with primary or metastatic carcinoma cells. Plasma cells or myeloma cells may have weak expression or be negative for this antigen.

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.

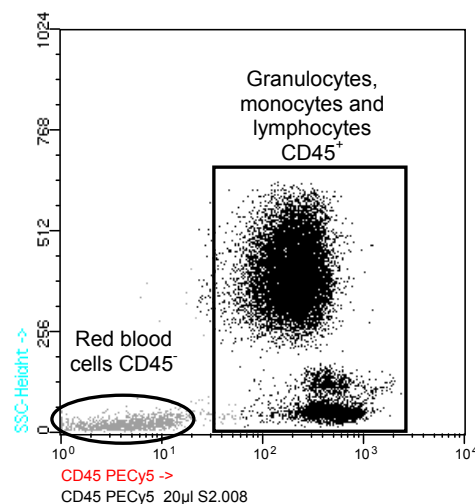
Preparation:

1. Transfer 100 µL of anticoagulated (EDTA) blood to a 12 x 75 mm polystyrene test tube (10⁶ cells).
2. Add 20 µL of CD45 PECy5 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 PECy5-conjugated antibody of the same isotype. **(Code No. ISOCNTPECy5IGG1).**
4. Incubate in the dark at room temperature at 4 °C for 30 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 betters 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 μ L of fluid.
9. Resuspend pellet in an appropriate fluid for flow cytometry, e.g. 0.3 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

Normal Peripheral Blood on a human donor



Cells were analyzed on a FACSCalibur (Becton Dickinson, San Jose, CA) flow cytometer, using Cell Quest acquisition software and PAINT-A-GATE. PRO, analysis software.

References:

1. Krensky AM, Sanchez-Madrid F, Robbins E, Nagy JA, Springer TA, Burakoff SJ. The functional significance, distribution, and structure of LFA-1, LFA-2, and LFA-3: cell surface antigens associated with CTL-target interactions. *J Immunol.* 1983;131:611-616
2. Escribano L, Orfao A, Villarrubia J, et al. Immunophenotypic characterization of human bone marrow mast cells: a flow cytometric study of normal and pathologic bone marrow samples. *Anal Cell Pathol.* 1998;16:151-159
3. Schwinzer R. Cluster report: CD45/CD45R. In: Knapp W, Dörken B, Gilks WR, et al, eds. *Leucocyte Typing IV: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 1989:628-634.
4. Jackson A. Basic phenotyping of lymphocytes: selection and testing of reagents and interpretation of data. *Clin Immunol Newslett.* 1990;10:49-55.

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