

Product: CD45 PURIFIED

Cat. Ref: 45PU1-0,1MG

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

Description: Monoclonal Mouse Anti-Human CD45 is recommended for use in flow cytometry for identification and analysis of CD45⁺ cells. The antibody is provided in liquid form in buffer containing 1% bovine serum albumin (BSA) (Lote: 113K1364 / SIGMA) and 0,09% NaN₃, pH 7.2.

HLDA: 4th International Workshops on Human Leucocyte Differentiation, WS Code CD 825.

Clone: D3/9

Isotype: IgG1

Fluorochrome: PURIFIED (pass through 0,22 µm filter)

Reactivity: The 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 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 and immunohistochemistry 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

Flow Cytometry Protocol:

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 10³ cells/µL and 200/ µL when the number of leukocytes is equal to 5 x 10³ 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. GOATPOLYFITCANTIMOUIGG) is added and the mixture is incubated at room temperature for 15

- 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 μ 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

References:

1. 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.**