

Product: CD45 Dy634
Cat. Ref: 45DY634-100T
Reagent provided: 100 test (20µl/test)
Description: Monoclonal Mouse Anti-Human CD45 is recommended for use in flow cytometry for identification and analysis of CD45+ cells. The conjugate is provided in liquid form in buffer containing Antibody Stabilizer® (Candor, Germany).
Clone: D3/9
Isotype: IgG1
HLDA: 4th International Workshops on Human Leucocyte Differentiation, WS Code 825.
Fluorochrome: DY-634 (Dyomics GmbH – *Colours of life*). Absorption/ emission max: 635nm/ 658 nm

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. Not 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/10P^{6P} 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 (10P^{6P} cells).
2. Add 20 µL of CD45 Dy634 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 Dy634-conjugated antibody of the same isotype. **(Code No. ISOCONTDY634IGG1).**
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 µL 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

References:

1. R Pulido and F Sanchez-Madrid. Biochemical nature and topographic localization of epitopes defining four distinct CD45 antigen specificities. Conventional CD45, CD45R, 180 kDa (UCHL1) and 220/205/190 kDa. The Journal of Immunology, Vol 143, Issue 6 1930-1936
2. Juan M. Zapata, Miguel R. Campanero, Monica Marazuela, Francisco Sanchez-Madrid, and Manuel O. de Landazuri. B-Cell Homotypic Adhesion Through Exon-A Restricted Epitopes of CD45 Involves LFA-1/ICAM-1, ICAM-3 Interactions, and Induces Coclustering of CD45 and LFA-1. Blood, Vol 86, No 5 (September 1), 1995: pp 1861-1872
3. Anne Marie-Cardine, Isabelle Maridonneau-Parini, Siegmund Fischer. Activation and internalization of p56lck upon CD45 triggering of Jurkat cells. European Journal of Immunology. Volume 24, Issue 6, Pages 1255 – 1261 Published Online: 1 Dec 2005
4. 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
5. 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

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