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ProductInformation

MONOCLONAL ANTI-HUMAN CD45RO
Clone UCHL1
Purified Mouse Immunoglobulin

Product Number **C 6555**

Product Description

Monoclonal anti-Human CD45RO (mouse IgG2a isotype) is derived from the UCHL1 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with influenza virus activated CA1 T-cell line.[1,2] The isotype is determined using the Sigma ImmunoType™ Kit (Prodcuct Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2).

Monoclonal anti-Human CD45RO recognizes the CD45RO, 180 kDa human cell surface glycoprotein. CD45RO is a single chain transmembraneous glycoprotein which represents the low molecular weight isoform of the Leucocyte Common Antigen (LCA).[3-8] It is expressed on most thymocytes, about 45% of peripheral blood T-cells, virtually all T-cells in skin reactive in filtrates and the majority of T-cell malignancies. It is also found on a subset of B-cells and on exceptional B-cell lymphomas. Natural killer (NK) cells do not express this antigen. Monocytes and granulocytes display CD45RO on their surfaces while tissue macrophages exhibit cytoplasmic expression. In granulocytes, CD45RO first appears at the blast/granulocyte stage. Mature granulocytes display a remarkable cytoplasmic pool of granule associated antigen.[9] T-cells expressing CD45RO antigen are T-memory cells or primed T-cells. CD4 positive cells carrying the CD45RO are good producers of helper signals, early producers of IL-2 and the main producers of IFN-γ. Changes in the levels of these cells have been reported for several clinical diseases, e.g. tuberculosis, leprosy, visceral leishmaniasis, rheumatoid arthritis, SLE, multiple sclerosis, and diabetes. The epitope recognized by monoclonal UCHL1 is sensitive to neuraminidase digestion, but not to formalin fixation, and paraffin embedding. This antibody is capable of detecting CD45RO antigen in tissue sections from material fixed by the following fixatives: unbuffered formol saline, neutral buffered formalin, zinc-formalin, Brunnell's primary fixative, Carnoy's fixative, and Methacarn. Trypsin digestion may enhance staining intensity in formalin fixed sections. The antibody detects CD45RO antigen in frozen sections, cell suspension, smears, imprints and cytospins.

Reagents

The product is provided as purified antibody (200 µg/ml) in 0.01 M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1% sodium azide as a preservative.

Precautions and Disclaimer

Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability

Store at 0-5 °C. Do Not Freeze.
If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Procedure

Indirect Immunofluorescent Staining

Reagents and Materials Needed but Not Supplied

1. a. Whole human blood collected by standard clinical blood evacuation tubes with EDTA, ACD-A or heparin anti-coagulant or
   b. Human cell suspension (e.g. peripheral blood mononuclear cells isolated on Histopaque®, Product Code 1077-1).
2. Diluent: 0.01 M PBS, pH 7.4, containing 1% BSA and 0.1% NaN_3, prechilled to 4 °C.

3. Fluorochrome (FITC, PE, or TRITC) conjugated anti-mouse secondary antibody diluted to recommended working dilution in diluent (Product No. F 2883 FITC Sheep anti-Mouse IgG (whole molecule), $F(ab')_2$ Fragment of Affinity Isolated Antibody is recommended). Aggregates in conjugates should be removed by centrifugation immediately prior to use.
4. Isotype matched non-specific mouse immunoglobulin (negative control, Product No. M 5409).
5. 12 x 75 mm test tubes.
6. Adjustable micropipet.
7. Refrigerated centrifuge.
8. Counting chamber.
9. Trypan blue (Product No. T 0776), 0.2% in 0.01 M PBS, pH 7.4.
10. Whole blood lysing solution.
11. Flow cytometer or fluorescent microscope.

Procedure

Prechill medium, perform all staining steps on ice and in a refrigerated centrifuge (2-6 °C).

1. a. Use 100 µl of whole blood or
   b. Adjust cell suspension to 2 x 10^7 cells/ml in diluent. Cells should be >90% viable as determined by dye exclusion (e.g. trypan blue). For each sample, add 50 µl or 1 x 10^6 cells per tube.
2. Prepare dilutions of Monoclonal anti-Human CD45RO in diluent to give a concentration of 20 µg/ml, i.e. 1:10 dilution of antibody. The CD45RO antibody is provided at 200 µg/ml.
3. Add 50 µl of diluted monoclonal antibody to tube(s) containing cells to be stained, i.e. 1 µg of monoclonal antibody per 1 x 10^6 cells in a final volume of 100 µl. Tap tube gently to mix. Incubate the cells on ice for 30 minutes.
   Proper controls to be included for each sample are:
   a. An autofluorescence control: 50 µl diluent in place of secondary antibody and monoclonal antibody.
   b. A negative staining control: 50 µl isotype-matched non-specific mouse immunoglobulin at a concentration of 20 µg/ml.
4. Pellet cells by centrifugation at 500 x *g*, 2-6 °C, for 10 minutes.
5. Remove supernatant by careful aspiration.
6. Resuspend cells in 2 ml diluent.
7. Pellet cells as in step 4 and repeat washing procedure (steps 5-6) twice.
8. After the last wash, resuspend the cells in 50 µl of the fluorochrome conjugated second antibody (diluted in diluent containing BSA) at the recommended concentration, except for autofluorescence controls. Resuspend cells for autofluorescence controls in diluent. Incubate on ice for 30 minutes. Protect from light at this and all subsequent steps. Note: If whole blood is used, use lysing solution after incubation according to manufacturer's instructions, then pellet and wash cells as in steps 4-6 twice, and proceed to step 10.
9. Centrifuge and wash as in steps 4-6 twice. Keep cells cold until analyzed.
11. After last wash, resuspend cells in 0.5 ml of diluent and analyze in a flow cytometer according to manufacturer's instructions.

It is advisable to run the appropriate negative controls. Negative controls establish background fluorescence and non-specific staining of the primary and secondary antibodies. The ideal negative control reagent is a mouse monoclonal or myeloma protein. It should be isotype-matched to the primary antibody, not specific for human cells and of the same concentration as the primary antibody. The degree of autofluorescence or negative control reagent fluorescence will vary with the type of cells under study and the sensitivity of the instrument used.

For fluorescence analysis of cells with Fc receptors, the use of isotype matched negative control is mandatory. In some systems it maybe necessary to incubate the monoclonal antibody and cells (at step 4) in 10-20% normal serum from the second antibody host species in order to decrease non-specific staining with the conjugated second antibody.

Product Profile

When assayed by flow cytometric analysis using 1 µg of the antibody to stain 1 X 10^6 cells, a fluorescence intensity is observed similar to that obtained with saturating monoclonal antibody levels. The percent population positive is also at the maximum percentage positive using saturating monoclonal antibody levels.

Monoclonal anti-Human CD45RO may be used for:

1. Identification, quantification and monitoring of helper/inducer and memory cytotoxic T-cells in peripheral blood, biological fluids, lymphoid organs and other tissues.
2. Analysis of T-cell activation.
3. Characterization of T-cell leukemias and lymphomas.
4. Studies of T-cells in health and disease.
5. Isolation, enrichment or depletion of helper/inducer or memory cytotoxic T-lymphocytes.
6. Immunohistological and immunocytochemical identification of normal and malignant T-lymphocytes (including formalin fixed, paraffin embedded tissue sections).
7. Immunoprecipitation of the 180 kDa, CD45RO antigen

Note: In order to obtain best results in different preparations, it is recommended that each individual user determine their optimum working dilutions by titration assay.

References

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