SIGMA®
CHEMICAL COMPANY

Product No. F-6023
Lot 017H4803

Monoclonal Anti-Human CD45RO
FITC Conjugate
Purified Mouse Immunoglobulin
Clone UCHL-1

Monoclonal Anti-Human CD45RO (mouse IgG2a isotype) is derived from the UCHL-1 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 Sigma ImmunoType™ Kit (Sigma Stock No. ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Sigma Stock No. ISO-2). The product is prepared by conjugation of fluorescein isothiocyanate (FITC) Isomer I to purified CD45RO monoclonal antibody. The conjugate is then purified by gel filtration to remove unbound FITC, no free FITC is detectable. The product is provided as purified antibody (200 µg/ml) in 0.01 M phosphate buffered saline pH 7.4 containing 1% BSA and 0.1% sodium azide as a preservative.

Description

Monoclonal Anti-Human CD45RO antibody recognizes the CD45RO 180 kD 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 infiltrates 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. UCHL-1 carrying CD4 positive cells 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 UCHL-1 is sensitive to neuraminidase digestion, but not to formalin fixation and paraffin embedding. The 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 also detects CD45RO antigen in frozen sections, cell suspension, smears, imprints and cytospins.

F/P Molar Ratio: 6.3

Performance

When assayed by flow cytometric analysis, using 10 µl 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.

Uses

Monoclonal anti human CD45RO FITC conjugate may be used for:

1. Identification, quantification and monitoring of helper/inducer 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 primed/memory T cells in health and disease.

Storage

Store at 2-8°C. Protect from prolonged exposure to light. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

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

*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.

Procedure for Direct 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 anticoagulant **or**
   b. Human cell suspension (e.g., peripheral blood mononuclear cells isolated on HISTOPAQUE® (Sigma Product No. 1077-1)).
2. Diluent: 0.01M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1% NaN_3.
3. FITC conjugated, isotype-matched, non-specific mouse immunoglobulin (negative control, Sigma Product No. F-6522).
4. 12 x 75 mm test tubes.
5. Adjustable micropipet.
6. Centrifuge.
7. Counting chamber.
8. Trypan blue (Sigma Product No. T-0776), 0.2% in 0.01 M PBS, pH 7.4.
9. 2% paraformaldehyde in PBS.
10. Whole blood lysing solution.
11. Flow cytometer.

Procedure

1. a. Use 100 µl of whole blood **or**
   b. Adjust cell suspension to 1 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 100 µl or 1 x 10^6 cells per tube.
2. Add 10 µl of conjugate to tube(s) containing cells to be stained. Vortex tube gently. Incubate the cells at room temperature (18 - 22°C) for 30 minutes. Proper controls to be included for each sample are:
   a. An autofluorescence control: 10 µl diluent in place of monoclonal antibody, followed by steps 3 - 7.
   b. A negative staining control: 10 µl of FITC conjugated, isotype-matched non-specific mouse immunoglobulin (Sigma Product No. F-6522) at the same concentration as test antibody followed by steps 3 - 7.
3. a. If whole blood is used, use lysing solution after incubation and wash cells according to manufacturer's instructions.
   b. If a mononuclear cell suspension is used, proceed to Step. 4.
4. Add 2 ml of diluent to all tubes.
5. Pellet cells by centrifugation at 500 x G for 10 minutes.
6. Remove supernatant by careful aspiration.
7. Resuspend cells in 0.5 ml of 2% paraformaldehyde. Analyze in a flow cytometer according to manufacturer's instructions.

Quality Control

It is advisable to run the appropriate negative controls. Negative controls establish background fluorescence and non-specific binding of the primary and secondary antibodies. The ideal negative control reagent is a mouse monoclonal or myeloma protein which has no reactivity with human cells. It should be isotype-matched to the antibody and of the same concentration and F/P molar ratio as the 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.

References

1. Leucocyte Typing III, Oxford University Press, pp 225-7, 800 (1987).
2. Smith, S., et al., Immunology, **58**, 63 (1986).
3. Norton, A., et. sl., J. Clin. Pathol. **39**, 399 (1986).
4. Norton, A., Isaacson, P.G., Am. J. Pathol., **128**, 225 (1987).
5. Linder, J., et. al., Am. J. Pathol., **127**, 1 (1987).
6. Terry, L., et. al., Immunology, **64**, 331 (1988).
7. Dohlsten, M., et. al., Eur. J. Immunol., **18**, 1173 (1988).
8. Cillari, E., et. al., J. Immunol., **146**, 1026 (1991).
9. Caldwell, C., et. al., Am. J. Clin. Pathol., **95**, 180 (1991).

Sigma warrants that its products conform to the information contained in this and other Sigma publications. Purchaser must determine the suitability of the products for its particular use. See reverse side of invoice or packing slip for additional terms and conditions of sale. Issued 02/97.