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Product Information

Monoclonal Anti-CD45RO-Quantum Red ™
clone UCHL-1
produced in mouse, purified immunoglobulin

Catalog Number **R4263**

Product Description

Monoclonal Anti-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 product is prepared by conjugation of Quantum Red with purified CD45RO monoclonal antibody. Quantum Red is Sigma's tandem fluorochrome in which a small organic dye, Cy5, is covalently linked to R-Phycoerythrin (PE). The PE absorbs light energy at 488 nm and emits in the excitation range of Cy5 which acts as the acceptor dye. The complex then emits at 670 nm. The conjugate is purified by gel filtration to remove unbound Quantum Red and antibody. No free Quantum Red or free antibody is detectable.

Monoclonal Anti-CD45RO antibody 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 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, 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.

Reagent

Supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA with 15 mM sodium azide as a preservative.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

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.

Product Profile

Assayed by flow cytometric analysis using 10 µl of the conjugate to stain 1 x 10^6 cells. Maximum signal to background and maximum percent positive are obtained.

Uses

Quantum Red Conjugated Monoclonal Anti-Human CD45RO 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.

Procedure

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 - peripheral blood mononuclear cells isolated with HISTOPAQUE®, Catalog No. 10771.
2. Diluent: 0.01 M Phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1% NaN_3.
3. Quantum Red conjugated, isotype-matched, non-specific mouse immunoglobulin (negative control, Catalog No. R 2263).
4. 12 x 75 mm test tubes.
5. Adjustable micropipette.
6. Centrifuge.
7. Counting chamber.
8. Trypan blue, Catalog No. 302643, 0.2% in 0.01 M PBS, pH 7.4.
9. 2% paraformaldehyde in PBS.
10. Whole blood lysing solution.
11. Flow cytometer.

Staining 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 Quantum Red conjugated, isotype-matched non-specific mouse immunoglobulin (Catalog No. R 2263) 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.

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

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