SIGMA®
CHEMICAL COMPANY

Product No. C-1813
Monoclonal Anti-Human CD45R
Purified Mouse Immunoglobulin
Clone KD3

Lot 073H4819

Monoclonal Anti-Human CD45R (mouse IgG1 isotype) is derived from the KD3 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from Balb/c mice immunized with human mononuclear cells.[1] 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 provided as purified antibody (200 µg/ml) in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA with 0.1% sodium azide (see MSDS)* as a preservative.

Description

The CD45 Common Leukocyte Antigen family is comprised of related single-chain transmembraneous glycoproteins of at least four isoforms which share a common large intracellular domain. The different isoforms are produced by alternative messenger RNA splicing of three of the extracellular exons of a single gene on chromosome 1. Their extracellular domains are heavily glycosylated. The intracellular domain has protein tyrosine phosphatase activity which may play an essential role in transmembrane signal delivery. Subsets of mature T cells express various combinations of all four isoforms, while virgin thymocytes express only the 180 kD isoform. Unstimulated T cells express the 220 kD isoform, while memory T cells express the 180 kD isoform, but not the 220 kD isoform. B cells predominantly express the 205 kD and 220 kD isoforms. Members of the CD45R subfamily are restricted to 180 kD (CD45RO), 220 kD (CD45RA), or 220, 205 and 190 kD (CD45RB). KD3 retains the CD45R designation because reactivity testing with a panel of appropriate transfectants has not yet been done. The KD3 antibody reacts in immunoblotting, and does not bind rabbit or human complement. The antibody is capable of detecting CD45R antigen in tissue sections from material fixed by the following fixatives: unbuffered formol saline, neutral buffered formalin, zinc-formalin, Brunnell's primary fixative, Bouin's solution and Methacarn. Trypsin digestion is usually needed when using formalin-fixed sections. The antibody also detects CD45R antigen in acetone-fixed frozen sections, cell suspension, smears, imprints and cytospins.

Performance

1. When assayed by flow cytometric analysis, 5 µl of the monoclonal antibody and a fluorescently labelled secondary antibody will stain 1 x 10^6 cells with a fluorescence intensity and percent positive that is similar to that observed with saturating amounts of monoclonal antibody.
2. A working dilution of 1:10 was obtained by indirect immunohistology using formalin-fixed, paraffin-embedded human tonsil.

Uses

Monoclonal Anti-Human CD45R antibody may be used for:
1. Identification, quantification and monitoring of CD45R carrying cells.
2. Immunohistochemical characterization of leukemias and lymphomas.

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.

Storage

Store at 0-5°C. Protect from prolonged exposure to light. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

*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 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 anticoagulant **or**
   b. Human cell suspension (e.g. peripheral blood mononuclear cells isolated on HISTOPAQUE® Sigma Stock No. 1077-1).

Reagents and Materials Needed but Not Supplied(cont.)

2. Diluent: 0.01M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1% NaN_3.
3. Fluorochrome (FITC, PE, or Quantum Red™) conjugated anti-mouse secondary antibody diluted to recommended working dilution in diluent (e.g. Sigma Product No. F-2883 FITC-Sheep Anti-Mouse IgG (whole molecule), $F(ab')_2$ fragment of Affinity Isolated Antibody). Aggregates in conjugates should be removed by centrifugation immediately prior to use.
4. Isotype-matched, non-specific mouse immunoglobulin (negative control, Sigma Product No. M-5284).
5. 12 x 75 mm test tubes.
6. Adjustable micropipet.
7. Centrifuge.
8. Counting chamber.
9. 0.2% Trypan blue (Sigma Product No. T-0776) in 0.01 M phosphate buffered saline, pH 7.4.
10. 2% paraformaldehyde in PBS.
11. Whole blood lysing solution.
12. 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 5 µl of monoclonal antibody to tube(s) containing cells to be stained. Vortex tube gently to mix. Incubate the cells at room temperature (18 - 22°C) for 30 minutes.
   Proper controls to be included for each sample are:
   a. Autofluorescence control: 5 µl diluent in place of monoclonal antibody.
   b. Negative staining control: 5 µl isotype-matched non-specific mouse immunoglobulin at the same concentration as test antibody.
3. After 30 minutes, add 2 ml of diluent to all tubes.
4. Pellet cells by centrifugation at 500 x G, for 10 minutes.
5. Remove supernatant by careful aspiration.
6. Resuspend cells in 2 ml diluent.
7. Repeat washing procedure (steps 4-6) twice.
8. After the last wash, resuspend the cells in 100 µl of the fluorochrome conjugated secondary antibody at the recommended concentration. For the autofluorescence control, add 100 µl of diluent. Incubate at room temperature (18 - 22°C) 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.
10. After last wash, resuspend cells in 0.5 ml of diluent or 2% paraformaldehyde (if cells are stored before analyzing) and 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 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 may be necessary to incubate the cells in 10-20% normal serum from the second antibody host species (at step 2 before adding monoclonal antibody) in order to decrease non-specific staining with the conjugated second antibody.

References

1. Ando, I., Tissue Culture and Res., (ed. P. Rohlich), p. 241, Academic Press, New York (1984).
2. Pulido, R., and Sanchez-Madrid, F., J. Immun., **143**, 1930 (1989).
3. Thomas, M., Ann. Rev. Immunol., **7**, 339 (1989).
4. Leucocyte Typing IV, Oxford University Press, pp 630, 640 (1989).

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