SIGMA®
CHEMICAL COMPANY

Product No. F-1527
Monoclonal Anti-Human CD45RA
FITC Conjugate
Purified Mouse Immunoglobulin
Clone F8-11-13

Lot 014H8930

Monoclonal Anti-Human CD45RA (mouse IgG1 isotype) is derived from the F8-11-13 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized mouse. Fresh human lymph node lymphocytes depleted of surface immunoglobulin positive cells were used as the immunogen. The isotype is determined using Sigma Immuno-Type™ Kit (Sigma Stock No. ISO-1) and by a double diffusion assay 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 CD45RA monoclonal antibody. The conjugate is then purified by gel filtration to remove unbound FITC, no free FITC is detectable. The conjugate is provided (4 µg/ml) as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA with 0.1% sodium azide (see MSDS)* as a preservative.

Description

FITC Conjugated Monoclonal Anti-Human CD45RA recognizes the 220 kD high molecular weight isoform of leukocyte common antigen. This antigen is a single-chain glycoprotein which is expressed on B cells, monocytes, and T cell subsets.

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.

F/P Molar Ratio: 5.2

Uses

FITC Conjugated Monoclonal Anti-Human CD45RA may be used for:

1. Identifying B cells in immunohistology.
2. Typing of lymphomas in paraffin sections.

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 sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

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.

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.

Reagents and Materials Needed but Not Supplied (cont.)

3. FITC conjugated, isotype-matched, non-specific mouse immunoglobulin (negative control, Sigma Product No. F-6397).
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 phosphate buffered saline, 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 1×10^7 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 1×10^6 cells per tube.
2. Add 10 µl of conjugate 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. An autofluorescence control: 10 µl diluent in place of monoclonal antibody followed by steps 3 - 8.
   b. A negative staining control: 10 µl of FITC conjugated, isotype-matched non-specific mouse immunoglobulin at the same concentration as test antibody followed by steps 3 - 8.
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 3-6) twice.
   Note: If whole blood is used, use lysing solution after incubation according to manufacturer's instructions, then repeat steps 3-6 twice, and proceed to step 8.
8. 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 (at step 2 before adding monoclonal antibody) in 10-20% normal serum from the second antibody host species in order to decrease non-specific staining with the conjugated second antibody.

References

1. Cobbold, S., et al., Leucocyte Typing III, 789 (1987).
2. Streuli, M., et al., J. Immunol., **141**, 3910 (1988).
3. Schwinzer, R., Leucocyte Typing IV, 628 (1989).
4. Dalchau, R., et al., Leucocyte Typing III, 814 (1987).
5. Dalchau, R., et al., Eur. J. Immunol. **16**, 993 (1986).

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