SIGMA®
CHEMICAL COMPANY

MONOCLONAL ANTI-MOUSE CD45
FITC CONJUGATE CLONE I3/2
Purified Rat Immunoglobulin

Product No. **F7900**

Monoclonal Anti-Mouse CD45 (rat IgG2a isotype) is derived from the M1-9.3HL hybridoma produced by the fusion of the NS1 murine cell line with splenocytes from a Lou/Ws1/M rat immunized with mouse thymocytes and spleen cells. The product is provided as purified antibody conjugated to fluorescein isothyocyanate isomer I. The conjugate is purified by gel filtration to remove unconjugated FITC and antibody. No free FITC or antibody is detectable. The product is provided at a concentration of 0.25 mg/ml in 0.01 M phosphate buffered saline, pH 7.2, containing 1% BSA, 2 mM EDTA and 0.1% sodium azide (see MSDS)* as a preservative.

Description
FITC Conjugated Monoclonal Anti-Mouse CD45 recognizes the 200 kD isoform of CD45 expressed on all leukocytes. Other isoforms are a 220 kD antigen (B220) expressed on pre-B cells and a 205 kD antigen expressed on macrophages. M1-9.3HL antibody recognizes CD45 on all mouse strains tested.

Performance
When assayed by flow cytometry, approximately 1 µg (4 µl) of the product will stain 1 x 10^6 mouse spleen cells to maximum fluorescence intensity and percent positive. Prior to adding the product to cells, it is recommended that cell surface Fc receptors be blocked by incubating the cells with 10% - 20% normal goat serum (Sigma Product No. G9023) for 10 minutes at 4°C. A stabilizing protein such as 1% BSA should be included in the diluent when making dilutions of this product.

F/P Molar Ratio: 3.0-6.0

Uses
FITC Conjugated Monoclonal Anti-Mouse CD45 may be used for:
1. Direct immunofluorescence analysis of mouse leukocytes by flow cytometry or fluorescence microscopy.
2. Simultaneous multicolor analysis when used in conjunction with R-PE or Quantum Red™ conjugates.

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.

* 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 hazardous and safe handling practices.

Procedure for Direct Immunofluorescent Staining of Splenocytes or Thymocytes
Reagents and Materials Needed but not Supplied
1. Diluent: 0.01 M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1% NaN_3.
2. FITC conjugated, isotype-matched, non-specific rat or mouse immunoglobulin (Sigma Product No. F6522).

Procedure
Notes:
1. In order to obtain best results in different preparations, it is recommended that each individual user determine their optimum dilution of antibody by titration assay.
2. Flow cytometric analysis of rodent cells yield better results when the cells are kept cold. Therefore, pre-chill all buffers and diluents, and keep the cells on ice during preparation and staining steps.

1. 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, Sigma Product No. T0776). For each sample, add 100 µl or 1 x 10^6 cells per tube. (Note: If cell preparation contains red blood cells, they can be lysed by incubating the cells in approximately 10 mls of 0.017 M Tris, 0.75% NH_4Cl, pH 7.2, at room temperature for 5 - 10 minutes followed by centrifugation and wash

ing 2 times in diluent).

2. Add 1 µg (4 µl) of monoclonal antibody to tube(s) - containing cells to be stained. Vortex tube gently to mix. Incubate the cells at 4°C for 30 minutes. Proper controls to be included for each sample are:
   a. Autofluorescence control: diluent in place of monoclonal antibody, followed by steps 3 - 8.
   b. Negative staining control: FITC conjugated, isotype-matched, non-specific rat or mouse immunoglobulin at the same concentration as test antibody, followed by steps 3 - 8.
3. After 30 minutes, add 2 ml of cold 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 of cold diluent.
7. Repeat washing procedure (steps 4-6) twice.
8. After last wash, resuspend cells in 0.5 ml of diluent or 2% paraformaldehyde if cells are stored before analyzing.

Quality Control

It is advisable to run the appropriate negative controls. Negative controls establish background fluorescence and non-specific staining of the primary and/or secondary antibodies. The best negative control reagent is a FITC conjugated, isotype-matched, rat or mouse monoclonal antibody or myeloma protein. It should not be reactive with the cells being analyzed and should be used at the same concentration as the fluorophore conjugated specific 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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