Product No. F-0292
Lot 095H4840

Monoclonal Anti-Mouse Kappa Light Chains
FITC Conjugate

Purified Antibody
Clone EM-34.1

Monoclonal Rat Anti-Mouse Kappa Light Chains (rat IgG2b isotype) is derived from the EM-34.1 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized F344 rat. Purified murine monoclonal immunoglobulins bearing kappa light chains were used as the immunogen.[1] The isotype was determined by radial immunodiffusion. Purified immunoglobulin from ascites fluid produced in SCID (Severe Combined Immuno-Deficient) mice is conjugated to fluorescein isothiocyanate (FITC) and further purified to remove unconjugated FITC. The conjugate is provided as a solution (150 µg/ml) in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 0.1% sodium azide (see MSDS)* as a preservative.

Specificity

FITC Conjugated Monoclonal Anti-Mouse Kappa Light Chains recognizes an epitope located in the kappa light chain of the mouse immunoglobulin molecule.[1] It does not cross-react with mouse lambda light chain cell surface immunoglobulins.

Description

Immunoglobulins are symmetrical molecules made up of two identical heavy chains and two identical light chains. There are two types of light chains, κ and λ. Each immunoglobulin molecule contains either κ or λ light chains. In the mouse, there is only one κ light chain class, but there are three λ chain classes (λ1-λ3). More than 95% of immunoglobulins (Ig) in most inbred strains of mice carry the κ type of light chains.[2] Anti-mouse antibodies are commonly produced by xenogeneic immunization of rabbits, goats or sheep, resulting in antibodies that cross-react with other immunoglobulins of other species, unless extensively adsorbed. FITC conjugated monoclonal anti-mouse immunoglobulins which are devoid of any binding capacity to human and many other species can therefore serve as an essential tool in many immunocytochemical and immunohistochemical applications.

F/P Molar Ratio: 8.0

Performance

When assayed by flow cytometric analysis, using 10 µl of the antibody to stain 1 x 10^6 mouse spleen cells, a fluorescence intensity and percent positive is observed similar to that obtained with saturating monoclonal antibody levels. 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. G-9023) in 1% BSA containing PBS for 10 minutes at 2-8°C.

Uses

FITC Conjugated Monoclonal Anti-Mouse Kappa Light Chains may be used for:

1. Identification and clonality assessment of normal, leukemic and lymphoma B cells in smears, cytospins and frozen sections.
2. Enumeration of kappa light chain expressing B lymphocytes in peripheral blood or tissues.
3. Studies of Fc receptor, kappa immunoglobulin binding cells.
4. Indirect immunofluorescent staining of human cells and tissues.

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 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. Mouse lymphoid cell suspension (e.g., spleen, thymus or lymph node)
2. Diluent: 0.01 M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1% NaN_3.
3. FITC conjugated, isotype-matched, non-specific rat immunoglobulin.
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. 10% Normal Goat Serum (Sigma Product No. G-9023) in diluent
10. 2% paraformaldehyde in PBS
11. Flow cytometer or fluorescent microscope.

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.
3. Step 4 is a cell surface Fc receptors blocking procedure.

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. T-0776). (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 washing 2 times in diluent).
2. Pellet cells by centrifugation at 200 x G for 10 minutes.
3. Remove supernatant by careful aspiration.
4. Resuspend cells in 10% normal goat serum and incubate at 4°C (on ice) for 10 minutes.
5. Repeat steps 2 - 3.
6. Resuspend cells in initial volume of diluent.
7. For each sample, add 100 µl or 1 x 10^6 cells per tube.
8. Add 10 µl of FITC conjugated monoclonal antibody to tube(s) containing cells to be stained. Vortex tube gently to mix. Incubate the cells at 4°C (on ice) for 30 minutes.
   Proper controls to be included for each sample are:
   a. Autofluorescence control: diluent in place of monoclonal antibody, followed by steps 9 - 14.
   b. Negative staining control: FITC conjugated, isotype-matched, non-specific rat immunoglobulin at the same concentration as test antibody, followed by steps 9 - 14.
9. After 30 minutes, add 2 ml of cold diluent to all tubes.
10. Pellet cells by centrifugation at 200 x G for 10 minutes.
11. Remove supernatant by careful aspiration.
12. Resuspend cells in 2 ml of cold diluent.
13. Repeat washing procedure (steps 10-12) twice.
14. 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 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

1. Baniyash, M., and Eshhar, Z., Eur. J. Immunol., **14**, 799 (1984).
2. Bothwell, A., et al., Nature, **298**, 380 (1982).

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