**Product No. F-1793**
**Monoclonal Anti Mouse Kappa and Lambda Light Chains**
**FITC Conjugate**
Purified Rat Immunoglobulin
Clones EM-34.1 and 9A8

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 κ light chains were used as the immunogen.[1] Monoclonal Rat Anti-Mouse Lambda Light Chains (rat IgG1 isotype) is derived from the 9A8 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized Wistar rat. Purified murine myeloma protein M315 was used as the immunogen.[2] The monoclonal antibodies are purified from ascites fluid, produced in SCID (Severe Combined Immuno-Deficient) mice. The product is a solution of fluorescein-labeled purified monoclonal antibody, prepared by conjugation of fluorescein isothiocyanate isomer I to purified Monoclonal Anti-Mouse Kappa Light Chains and to Monoclonal Anti-Mouse Lambda Light Chains. The product is purified by gel filtration and contains no detectable free FITC. The product is provided as a solution (100 µg/ml of each monoclonal antibody) in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 0.1% sodium azide (see MSDS)* as a preservative.

**Specificity**

Monoclonal Anti-Mouse Kappa Light Chains recognizes an epitope located in the κ light chain of the mouse immunoglobulin molecule.[1] Monoclonal Anti-Mouse Lambda Light Chains recognizes an epitope located in the Vλ2$^{315}$ domain of the mouse immunoglobulin molecule; this V (variable) domain epitope is also expressed on λ1 and λ3 chains.[2] It detects 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.[3] About 5% of normal immunoglobulin (Ig) in most inbred strains of mice carry the λ type of light chains, of which λ1 comprises about 80% and λ2, λ3 the remaining 20%.[2,3] 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. 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.

**Uses**

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

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

**Antibody Performance**

When assayed by flow cytometric analysis using 10 µl of the antibody to stain 1 x $10^6$ mouse spleen cells maximum fluorescence intensity and percent population positive are obtained. 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.

**Storage**

For continuous use, store at 2-8°C. For extended storage, freeze in working aliquots. Repeated freezing and thawing is **not** recommended. Storage in "frost-free" freezers is **not** recommended. 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.

**Direct Immunofluorescent Staining of Mouse Splenocytes or Thymocytes by Monoclonal Rat FITC Conjugated Antibodies**

Reagents and Materials Needed but not Supplied

1. Mouse lymphoid cell suspension (e.g. spleen, thymus and lymph node).
2. Diluent: 0.01 M PBS, pH 7.4, containing 1% BSA (Sigma Product No. P-3688) and 0.1% $NaN_3$ (prechilled).
3. FITC conjugated isotype matched non-specific rat or mouse immunoglobulin (negative control).
4. 12x75 mm test tubes.
5. Adjustable micropipet.
6. Centrifuge.
7. Counting chamber.
8. Trypan blue (Sigma Product No. T-0776), 0.2% in 0.01M PBS, pH 7.4.
9. 2% paraformaldehyde in PBS.
10. Normal Goat Serum (Sigma Product No. G-9023) diluted to 10% in diluent
11. Flow cytometer or fluorescent microscope.

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).
2. Pellet cells by centrifugation at 200 x g for 10 minutes. Remove supernatant by careful aspiration.
3. Resuspend cells with 10% normal goat serum and incubate for 10 minutes at melting ice temperature.
4. Repeat step 2.
5. Resuspend cells in initial volume of diluent.
6. For each sample add 100 µl or $1x10^6$ cells per tube.
7. Add 10 µl FITC conjugated monoclonal antibody to tube(s) containing cells to be stained. Vortex tube gently to mix. Incubate the cells at melting ice temperature for 30 minutes. Protect from light at this and all subsequent steps.

   Proper controls to be included for each sample:
   a. An autofluorescence control: 10 µl diluent in place of FITC conjugated antibody followed by steps 8-13.
   b. A negative staining control: 10 µl of FITC conjugated isotype matched non-specific mouse or rat immunoglobulin at the same concentration as test antibody followed by steps 8-13.
8. After 30 minutes add 2 ml of cold diluent to all tubes.
9. Pellet cells by centrifugation at 200 x g for 10 minutes.
10. Remove supernatant by careful aspiration.
11. Resuspend cells in 2 ml cold diluent.
12. Pellet cells as in step 9 and repeat washing procedure (steps 8-11) twice.
13. 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.

Notes

1. Step 3 blocks cell surface Fc receptors.
2. Flow cytometric analysis of rodent cells yields better results when the cells are kept cold. Therefore, pre-chill all buffers and diluent and keep the cells on ice during preparation and staining steps.

**Quality Control**

It is advisable to run the appropriate negative controls. Negative controls establish background fluorescence and non-specific staining of the primary antibodies. The ideal negative control reagent is an FITC conjugated rat monoclonal or myeloma protein. It should be isotype matched, F/P molar ratio matched, not specific for mouse cells and of the same concentration as the test 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 fluorescent analysis of cells with Fc receptors the use of isotype matched negative controls is mandatory

.**References**


1. Baniyash, M., and Eshhar, Z., Eur. J. Immunol., **14**, 799 (1984).
2. Bogen, B., Scand. J. Immunol., **29**, 273 (1989).
3. Bothwell, A., et al., Nature, **298**, 380 (1982).



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