3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone (800) 325-5832 (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

MONOCLONAL ANTI-MOUSE LAMBDA LIGHT CHAINS
Clone 9A8
FITC Conjugate
Purified Immunoglobulin

Product No. **F 0417**

Product Description

Monoclonal 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 the Wistar rat. Purified mouse myeloma protein M315 was 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.

FITC Conjugated Monoclonal Anti-Mouse Lambda Light Chains recognizes an epitope located in the V$\lambda 2^{315}$ domain of the mouse immunoglobulin molecule. This V (variable) domain epitope is also expressed on λ1 and λ3 chains.[1] The product detects mouse lambda light chain cell surface immunoglobulins. It does not cross-react with the mouse kappa light chain.

FITC Conjugated Monoclonal Anti-Mouse Lambda 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 lambda light chain expressing B lymphocytes in peripheral blood or tissues.
3. Studies of Fc receptor, lambda immunoglobulin binding cells.
4. Indirect immunofluorescent staining of human cells and tissues.

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). 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%.[1,2]

The mouse is extensively used as a research model in pharmacology, oncology and in studies of immunological systems. Mouse polyclonal and monoclonal are widely used as primary antibodies. Secondary antibodies to λ light chains are particularly valuable for the detection, quantification, isotyping and purification of mouse immunoglobulins expressing λ light chains. 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 serve as an essential tool in many applications.

Reagents

The conjugate is provided as a solution (100 µg/ml) in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 0.1% sodium azide as a preservative.

Precautions

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.

Product Profile

F/P Molar Ratio: 3 to 8

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 (Product No. G-9023) in 1% BSA containing PBS for 10 minutes at 2-8 °C (procedure attached).

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.

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 (Product No. T0776), 0.2% in 0.01 M PBS, pH 7.4.
9. 10% Normal Goat Serum (Product No. G9023) 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 receptor 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, 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 in diluent and incubate at 4 °C 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 for 30 minutes.
   Proper controls to be included for each sample are:
   a. Autofluorescence control: diluent in place of monoclonal antibody.
   b. Negative staining control: FITC conjugated, isotype-matched, non-specific rat immunoglobulin at the same concentration as test antibody.
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 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

1. Bogen, B., Scand. J. Immunol., **29**, 273 (1989).
2. Bothwell, A.L.M., et al., Nature, **298**, 380 (1982).

Pcs11/99