

Product No. A 1812
Lot 057H4894

Monoclonal Anti-FITC
Alkaline Phosphatase Conjugate
Immunoglobulin Fraction of Mouse Ascites Fluid
Clone FL-D6

Monoclonal Anti-FITC (mouse IgG1 isotype) is derived from the hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized mouse. A FITC-BSA conjugate was used as the immunogen. The isotype is determined using the Sigma ImmunoType[™] Kit (Sigma Stock No. ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Sigma Stock No. ISO-2). The immunoglobulin fraction of Mouse Monoclonal Anti-FITC is conjugated to Alkaline Phosphatase by protein cross-linking with 0.2% glutaraldehyde.¹ The product is supplied as a solution in 0.05 M Tris buffer, pH 8.0, containing 1% BSA, 1.0 mM MgCl₂, 50% glycerol and 15mM sodium azide (see MSDS)* as a preservative.

Specificity

In an ELISA, Alkaline Phosphatase Conjugated Monoclonal Anti-FITC will react with either free or conjugated FITC. The antibody does not react with bound or free TRITC (tetramethylrhodamine isothiocyanate). Monoclonal Anti-FITC recognizes the free FITC molecule and FITC conjugated to various biomolecules such as proteins (immunoglobulins, enzymes), oligonucleotides, nucleic acids and other ligands.

Total IgG Concentration: 1.4 mg/ml.

Description

FITC (fluorescein isothiocyanate) is a fluorochrome dye that absorbs ultraviolet or blue light causing molecules to become excited and emit a visible yellow-green light. This emission ceases upon removal of the light causing the excitation. Fluorochrome labeling provides rapid, accurate localization of antigen-antibody interaction when one of the reactants is part of a cell, tissue or other biological structure. FITC is a commonly used marker for antibodies in immunofluorescent techniques since the conjugation of FITC to proteins is relatively easy and does not, in general, destroy the biological activity of the labeled protein. FITC is widely used as a hapten to label different proteins. Antibodies to FITC are used

to identify FITC labeled proteins and as models to study the mechanism of antibody response to a well defined hapten. Antibodies to FITC serve as universal indicator reagents by bridging FITC with another immunohistochemical reagent such as alkaline phosphatase or horseradish peroxidase.

Uses

Monoclonal Anti-FITC may be used for the detection of FITC and as a universal indicator reagent for bridging FITC with other immunochemical reagents. It can be used in ELISA and immunofluorescent techniques. A FITC Anti-FITC system has been used in the amplification of signal in immunofluorescent detection and as a means of separating bound from free tracer by affinity chromatography. The antibody can also be used to isolate cells that have an FITC labeled ligand on their surface.

Working Dilutions

1. Indirect ELISA

A dilution of 1:30,000 was determined by indirect ELISA using microtiter plates coated with human IgG (5 µg/ml) and FITC Conjugated Monoclonal Anti-Human IgG, Fc Specific, (Sigma Product No. F 5016) as the primary antibody.

2. Dot Blot

- a. A dilution of 1:80,000 was determined in an indirect assay using 20 ng of human IgG/dot and FITC Monoclonal Anti-Human IgG, Fc specific, (Sigma Product No. F 5016) as the primary antibody.
- b. In an indirect chemiluminescence system using 20 ng human IgG/dot and FITC Monoclonal Anti-Human IgG, Fc specific, (Sigma Product No. F 5016) as the primary antibody, this product was determined to have a dilution of 1:80,000 when used as secondary antibody. 1,2-Dioxetane and enhancer was used as substrate.

3. Indirect Immunohistology

A dilution of 1:20 was determined using formalin-fixed, paraffin-embedded sections of human tonsil and FITC Monoclonal Anti-Human IgG, Fc specific, (Sigma Product No. F 5016) as the primary antibody.

* 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

Storage

Store at 2-8°C. **Do Not Freeze.**