



Product Information

MONOCLONAL ANTI-HUMAN IgG3 CLONE HP-6050 FITC CONJUGATE Immunoglobulin Fraction of Mouse Ascites Fluid

Product No. **F 4641**

Product Description

Monoclonal Anti-Human IgG3 (mouse IgG1 isotype) is derived from the HP-6050 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized mouse.¹ Purified myeloma human IgG3 covalently coupled to polyaminostyrene (PAS) microbeads was used as the immunogen. The isotype is determined using Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2). The immunoglobulin fraction of the ascites fluid is conjugated to fluorescein isothiocyanate (FITC) and then further purified to remove unconjugated FITC.

FITC Monoclonal Anti-Human IgG3 reacts specifically with human IgG3. No reactivity is observed with human IgG1, IgG2, or IgG4. This clone has been evaluated for specificity using a wide range of immunological techniques in the IUIS/WHO collaborative study and has been identified as one of the most applicable IgG3 specific antibodies.²

Human IgG consists of four subclasses (1-4) that can be recognized by antigen differences in their heavy chains. They constitute approximately 65, 30, 5, and 4% of the total IgG respectively. Each subclass has different biological and physiochemical properties and the IgG subclass may be preferentially produced to different antigens. For instance, anti-polysaccharide responses are mainly of the IgG2 subclass while protein antigens give rise to IgG1 and IgG3. Subclass G1 is the predominant subclass of *in vivo* and *in vitro* produced anti-tetanus toxoid antibodies. Only IgG1 and IgG3 are capable of adherence to mononuclear phagocytes while IgG2 and IgG4 autoantibodies are not associated with disorders like hemolytic anemia. Serum IgG subclass deficiencies have been recorded for different patient groups. For example, IgG2-IgG4 deficiency is associated with IgA deficiency as found in patients with ataxia telangiectasia. Low IgG2 levels were found in patients with SLE and juvenile diabetes mellitus.

Disproportionate elevation of IgG1 in CSF of patients with multiple sclerosis has also been described. Examination of the distribution pattern of IgG subclasses in different types of diseases may provide insight into the immunological processes involved, and may assist in the diagnosis of various disorders.

Reagents

The conjugate is provided as a liquid in 0.01 M phosphate buffered saline, pH 8.0, with 1% inactivated BSA and 15 mM sodium azide as a preservative.

Product Profile

1. A minimum working dilution of 1:64 was determined by fluorescent dot immunobinding assay (F-DIBA) using a 4-8 µg dot of human IgG3.
2. A minimum working dilution of 1:8 was determined by Particle Immunofluorescence Assay (PIFA) using a 50 µl suspension of Human IgG-Agarose coated with approximately 20 µg of human IgG.

In order to obtain best results, it is recommended that each individual user determine working dilutions by titration assay.

F/P Molar Ratio: 3 to 5

The F/P molar ratio is determined spectrophotometrically as follows:

$$F/P = \frac{A_{495} \times 1.4}{A_{280} - (0.36 \times A_{495})} \times 0.41$$

Where:

- 0.2 = The extinction coefficient of bound FITC at a concentration of 1 µg per ml at pH 7.2
- 0.36 = The fluorochrome absorbance correction factor (non-protein absorbance).
- 0.41 = The factor for conversion of fluorochrome to protein ratios from weight to molar ratios.

Storage and Stability

For continuous use, store at 2-8 °C for up to one month. For extended storage, solution may be frozen in working aliquots. Repeated freezing and thawing is not recommended. If slight turbidity occurs upon prolonged storage, clarify by centrifugation before use.

References

1. Reimer, C., et al., *Hybridoma*, **3**, 263, (1984).
2. Jefferies, R., et al., *Immunol. Lett.*, **10**, 223 (1985).
3. Stevens, R., et al., *J. Clin. Immunol.*, **3**, 65 (1985).
4. Van der Meulen, F., et al., *Brit. J. Haematol.*, **46**, 47 (1980).
5. Oxelius, V., *Amer. J. Med.*, **30/3**, 7 (1984).
6. Kaschka, W., et al., *Infext. Immun.*, **26**, 933 (1979).

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