Product No. A-0185
Lot 026H4840

Monoclonal Anti-Biotin Peroxidase Conjugate

Ig Fraction of Mouse Ascites Fluid
Clone BN-34

Monoclonal Anti-Biotin (mouse IgG1 isotype) is derived from the BN-34 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with biotinylated KLH. The isotype is determined using 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 the ascites fluid is conjugated to peroxidase by protein cross linking with 0.2% glutaraldehyde. The conjugated antibody is lyophilized from a solution of 1% BSA containing 0.01% thimerosal as preservative.

Uses

Biotin is an essential vitamin required for cells in living organisms and in cultures. The high binding affinity of biotin to egg white avidin or bacteria-derived streptavidin has been exploited in the design of immunoassays and immunohistologic staining techniques to serve as a basis for identifying antigen-antibody interactions. The most popular methods involve localization of the antigen with a primary antibody, addition of a biotinylated antibody to bind to primary antibody, application of avidin-enzyme (usually horseradish peroxidase) and reaction with a chromogenic substrate to localize the antibody-biotin-avidin-enzyme complex. While standard assay methods using the biotin-avidin-enzyme complex will suffice for most studies, there are occasions when enhanced sensitivity is needed to detect minute amounts of antigen or localize low densities of antigens in histologic sections. These conventional immunoassay methods are improved by the development of Monoclonal Anti-Biotin which enhances the sensitivity of avidin-biotin immunoassays by selectively enlarging the avidin-biotin-enzyme complex by bridging biotin to a second layer of avidin-biotin-enzyme complex, thus increasing the signal from substrate conversion. In addition, this antibody can be used in many other applications where biotin may be introduced as target label. For instance, it may be used in detection of low copy human papilloma virus DNA and mRNA in routine paraffin sections of cervix by sensitive non-isotopic, *in situ* hybridization. It has also been used successfully for detection of micro-injected, biotin-haptenized cytoskeletal proteins which enable direct examination of the pattern of incorporation and turnover of cytoskeletal proteins in living cells.

Specificity

Peroxidase Monoclonal Anti-Biotin reacts specifically with biotin conjugated to various proteins.

Molar Ratio (IgG:Peroxidase): 1.3:1.0

Enzyme Activity: 600 purpurogallin units/ml

Enzyme activity is determined using 5% pyrogallol (Sigma Product No. P-0381) in deionized water, pH 6.0, at 20°C. One purpurogallin unit will form 1 mg of purpurogallin from pyrogallol in 20 seconds at pH 6.0, 20°C.

Titers

1. 1:30,000 (Indirect ELISA)

Titer is defined as the dilution of conjugate sufficient to give a change in absorbance of 1.0 at 450 nm after 30 minutes of substrate conversion at 25°C (Voller, et al.)[1]. Microtiter plates are coated with purified human IgG at a concentration of 5 µg/ml in 0.05 M carbonate-bicarbonate buffer, pH 9.6 (carbonate-bicarbonate buffer capsules are available as Sigma Product No. C-3041). Biotinylated polyclonal or monoclonal anti-human IgG (i.e., Biotin Conjugated Monoclonal Anti-Human IgG (Fc specific), Clone HP-6017, Sigma Product No. B-3773) is used as the primary antibody.

Substrate: *o*-Phenylenediamine Dihydrochloride (OPD, Sigma Product No. P-8287), 0.4 mg/ml in 0.05 M phosphate-citrate buffer, pH 5.0 containing 0.03% sodium perborate (Phosphate-Citrate Buffer Capsules with Sodium Perborate are available as Sigma Product No. P-4922).

2. Dot Blot

   a. A dilution of 1:4,000 was determined in an indirect assay using human IgG (100-10 µg/dot). Biotinylated polyclonal or monoclonal anti-human IgG (i.e., Biotin Conjugated Monoclonal Anti-Human IgG (Fc specific), Clone HP6017, Sigma Product No. B-3773) is used as the primary antibody.
   b. In an indirect chemiluminescence system using ng 2.5 - 20 ng human IgG/dot and Biotinylated polyclonal or monoclonal anti-human IgG (i.e., Biotin Conjugated Monoclonal Anti-Human IgG (Fc specific), Clone HP6017, Sigma Product No. B-3773) as the primary antibody, this product was determined to have a dilution of 1:80,000 when used as secondary antibody. Luminol plus enhancer was used as substrate.

3. Immunohistology

A dilution of 1:300 was determined in an indirect assay using formalin-fixed, paraffin-embedded human tonsils. Biotinylated polyclonal or monoclonal anti-human IgG (i.e., Biotin Conjugated Monoclonal Anti-Human IgG (Fc specific), Clone HP6017, Sigma Product No. B-3773) is used as the primary antibody.

Working Dilutions

Working dilutions should be determined by titration assay. Due to differences in assay systems, these titers may not reflect the user's actual working dilution.

Reconstitution and Storage Instructions

To one vial of lyophilized powder, add 0.5 ml of deionized water. Rotate vial gently until powder dissolves. Prior to reconstitution store the product at 2-8°C. After reconstitution, the solution may be stored frozen in working aliquots. Repeated freezing and thawing is **not** recommended. If slight turbidity occurs upon prolonged storage clarify the solution by centrifugation before use.

Reference

1. Voller, A., et al., Bulletin WHO, **53**, 55 (1976).