Product Information

Monoclonal Anti-Biotin-Peroxidase Antibody Produced in Mouse

Clone BN-34, Purified immunoglobulin

A0185

Product Description

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 Mouse Monoclonal Antibody Isotyping Reagent (ISO2). The immunoglobulin fraction of the ascites fluid is conjugated to peroxidase by protein cross linking with 0.2% glutaraldehyde.

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

Reagent

- Supplied as a Lyophilized from 0.01 M phosphate buffered saline, pH 7.4, containing 0.05% MIT.
- Antibody concentration: 5-11 mg/mL
- Molar Ratio: 0.6-1.5 M

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses.

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 are not recommended. If slight turbidity occurs upon prolonged storage clarify the solution by centrifugation before use.

Product Profile

Indirect ELISA

Minimum 1:30,000 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.[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 C3041.

Biotinylated polyclonal or monoclonal anti-human IgG (for example, Monoclonal Anti-Human IgG (Fc specific)-Biotin, Clone HP-6017, B3773) is used as the primary antibody.

Substrate: *o*-Phenylenediamine Dihydrochloride (OPD), P8287, 0.4 mg/mL in 0.05 M phosphate-citrate buffer, pH 5.0 containing 0.03% sodium perborate. Phosphate-Citrate Buffer with Sodium Perborate capsules are available as P4922.

Immunoblotting

A working dilution of 1:80,000 is determined using immunoblot assay detecting GAPDH in total cell extract of HeLa cells.

Immunohistochemistry

A minimum dilution of 1:300 was determined in an indirect assay using formalin-fixed, paraffin-embedded human tonsils. Biotinylated polyclonal or monoclonal anti-human IgG (for example, Monoclonal Anti-Human IgG (Fc specific)-Biotin, Clone HP-6017, B3773), is used as the primary antibody.

Note: 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. Enzyme Activity: at least 200 purpurogallin units/mL Enzyme activity is determined using 5% pyrogallol, P0381, 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, at 20 °C.

Reference

1. Voller, A., et al., Bull. World Health Org., 53, 55 (1976).

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