

## Product Information

**MONOCLONAL ANTI-BIOTIN  
CLONE BN-34  
Alkaline Phosphatase Conjugate  
Immunoglobulin Fraction of Mouse Ascites Fluid**

Product No. **A 6561**

### 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 biotin-KLH. The isotype is determined using the 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 Alkaline Phosphatase using 0.2% glutaraldehyde.

Monoclonal Anti-Biotin recognizes the free biotin molecule and biotin conjugated to various immunoglobulins in ELISA and immunohistochemical techniques. Specificity was verified by using biotinylated goat antibodies reactive against human and rabbit antigens coated on microtiter plates.

Monoclonal Anti-Biotin may be used in a wide range of applications including blotting (Western, Southern and Dot Blots), immunohistology, *in situ* nucleic acids hybridization, ELISA, fluorescent activated cell-sorting (FACS) and electron microscopy.

Biotin is an essential vitamin required by cells in living organisms or in culture. The high binding affinity of biotin to egg white or bacteria-derived avidin has been exploited in the design of immunoassays and immunohistologic staining techniques. The most popular procedure involves localization of the antigen with a primary antibody, addition of a biotinylated antibody to bind to the primary antibody, application of avidin conjugated enzyme, and finally, reaction with a chromogenic substrate. While standard assay methods using the avidin-biotin-enzyme complex will suffice for most studies, there are occasions when enhanced sensitivity is needed to detect smaller amounts of antigen or localize low density antigens in histologic sections.

Conventional immunoassay methods are improved by the use of Monoclonal Anti-Biotin, which enhances the sensitivity of avidin-biotin immunoassays by adding a second layer of enzyme via the biotin specific monoclonal antibody. This antibody can be used in many other applications where biotin can be introduced as a target label. For instance, it has been used to aid in the 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 the detection of microinjected biotin-haptenized cytoskeletal proteins to examine directly the pattern of incorporation and turnover of cytoskeletal proteins in living cells.

### Reagents

The conjugate is supplied in 0.05 M Tris buffer, pH 8.0, containing 1% BSA, 1.0 mM MgCl<sub>2</sub>, 50% glycerol and 15 mM sodium azide as a preservative.

### Precautions

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 hazards and safe handling practices.

### Product Profile

1. The minimum working dilution by ELISA is determined by testing dilutions in microtiter plates coated with human IgG (5 µg/ml) using Biotin Monoclonal Anti-Human IgG (Fc specific) (Product No. B 3773) as the primary antibody.
2. a. A minimum working dilution of 1:10,000 is determined by indirect dot blot assay using 20 ng human IgG/dot and Biotin Monoclonal Anti-Human IgG (Fc specific) (Product No. B 3773) as the primary antibody.

b. In an indirect chemiluminescence system using 20 ng human IgG/dot and Biotin Monoclonal Anti-Human IgG (Fc specific) (Product No. B 3773) as the primary antibody, this product was determined to have a minimum working dilution of 1:10,000 when used as secondary antibody. 1,2-Dioxetane and enhancer was used as substrate.

3. A minimum working dilution of 1:20 was determined by indirect immunohistology assay using formalin-fixed, paraffin-embedded sections of human tonsil and Biotin Monoclonal Anti-Human IgG (Fc specific) (Product No. B 3773) as the primary antibody.

**Storage**

Store at 2-8 °C. Do Not Freeze.

Working dilution should be discarded if unused within 12 hours.

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