BOSTER BIOLOGICAL TECHNOLOGY
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888-466-3604 | support@bosterbio.com | www.bosterbio.com


# Mouse Anti-Rabbit IgG (H+L) Secondary Antibody, Biotin Conjugated

## Catalog number: BM2004

This package insert must be read in its entirety before using this product.

For research use only. Not for use in diagnostic procedures.

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antibody and ELISA experts

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**Catalog Number:** BM2004, **Storage:** At -20°C for one year from date of receipt. Avoid repeated freezing and thawing.

## Product Overview

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|---|---|
| Product Name | Mouse Anti-Rabbit IgG (H+L) Secondary Antibody, Biotin Conjugate |
| Synonyms | Biotin-conjugated Mouse Anti-Rabbit IgG; Mouse Anti-Rabbit IgG Biotinylated Antibody; Biotinylated Mouse Anti-Rabbit IgG Secondary Antibody; Mouse Anti-Rabbit IgG Secondary Antibody, Biotin-labeled |
| Description | Mouse Anti-Rabbit IgG (H+L) Secondary Antibody, Biotin Conjugate, for indirect sensitive immunodetection and/or quantification of low-abundance target proteins through ELISA or IHC by using reporter-labeled biotin-binding signal amplification systems. |
| Reagent Type | Biotin conjugated secondary antibody |
| Conjugate | Biotin |
| Host | Mouse |
| Target Species | Rabbit |
| Antibody Class | IgG |
| Clonality | Polyclonal |
| Immunogen | Whole molecule rabbit IgG |
| Purification | Immunoaffinity chromatography |
| Specificity | Rabbit IgG specific; no cross-reactivity with human/goat/bovine IgG |
| Form Supplied | Liquid: concentrated buffered stock solution |
| Formulation | 0.5 mg biotin-conjugated secondary antibody<br>0.01 M PBS (PH 7.4)<br>50% glycerol |
| Pack Size | 0.5 ml |
| Concentration | 1 mg/ml |
| Application | ELISA, IHC |
| Storage | At -20˚C for one year from date of receipt. Avoid repeated freezing and thawing. |
| Shipping | Ships in room temperature. Can also ship with gel ice or dry ice but not necessary. |
| Precautions | FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR CLINICAL USE |

## Assay Information

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| Sample Type | Human primary-antibody-probed: SDS-PAGE separated-, membrane-immobilized-proteins from cell/tissue lysates, formalin-fixed paraffin-embedded (FFPE) tissue sections on slides |
| Assay Type | Immunoanalytical |
| Technique | Indirect immunodetection of target protein via reporter-labeled biotin-binding detection systems |
| Assay Purpose | Protein detection/quantification |
| Equipment Needed | WB/Dot blot/ELISA/IHC instrumentation; Reporter signal detectors: X- |

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| | ray film cassette; a charge-coupled device (CCD) imager; Spectrophotometer; fluorescent or electron microscope |

## Main Advantages

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| Specificity | High signal-to-noise ratio |
| High Signal Amplification | Multiple secondary antibodies can bind to a single primary antibody; Multiple reporter molecules localize to a single biotin via avidin/streptavidin bridges |
| Fast | Fewer processing steps - no need to add a substrate; Less optimization required compared to enzymatic detection; Generates strong signals in a relatively short time span; Fluorescence can be observed directly |
| Quantifieable | Allows quantification of detected signal |
| Easy to Use | Supplied in a workable liquid format |
| Flexible | Biotin- (Strept)Avidin system can be coupled with various types of reporters (enzymes, fluorochromes, fluorophores, chromophores, etc.); One type of labeled secondary antibody can be used to recognize different types of primary antibodies of the target organism specific to a particular antigen |
| Compatible | Biotin does not interfere with catalysis or antibody binding |

## Background

Most commonly, secondary antibodies are generated by immunizing the host animal with a pooled population of immunoglobulins from the target species. The host antiserum is then purified through immunoaffinity chromatography to remove all host serum proteins, except the specific antibody of interest. Purified secondary antibodies are further solid phase adsorbed with other species serum proteins to minimize cross-reactivity in tissue or cell preparations, and are then modified with antibody fragmentation, label conjugation, etc., to generate highly specific reagents. Secondary antibodies can be conjugated to a large number of labels, including enzymes, biotin, and fluorescent dyes/proteins. Here, the antibody provides the specificity to locate the protein of interest, and the label generates a detectable signal. The label of choice depends upon the experimental application.

Biotinylated antibodies are widely used in systems where signal amplification is desired. Often 15-20 biotin moieties are coupled to a single IgG secondary antibody. Biotin binds avidin, streptavidin, or neutravidin with a high degree of affinity and specificity. In immunoassays avidin/streptavidin-biotin binding is used as a bridge between antibodies and reporters like enzymes (HRP, AP), fluorophores, chromophores, etc. Both avidin and streptavidin are tetrameric proteins capable of binding 4 biotin groups to each molecule of avidin or streptavidin, thus amplifying the signal intensity and detection sensitivity by increasing the concentration of reporters at the antigenic site. Two main biotin-binding detection systems have been widely utilized: Avidin-Biotin Complex (ABC) and Labeled Streptavidin Biotin (LSAB) methods. In the ABC method free avidin (or streptavidin) is used as a bridge/link between the biotinylated antibody and  biotinylated reporter molecule, resulting in three reporter molecules coupled to the biotinylated antibody. The LSAB method employs a reporter-labeled streptavidin (avidin or neutravidin can alternatively be used) to detect the bound biotinylated-secondary antibody on the tissue section, blotting membrane or ELISA plate, improving the sensitivity of detection by 8-fold. The LSAB method is used when the avidin-biotin-enzyme complex in the ABC method becomes too large to penetrate the tissue.

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