

Amersham Anti-rabbit IgG, biotinylated species- specific whole antibody (from donkey)

Product Specification Sheet

Code: RPN1004

Warning

For research use only.

Not recommended or intended for diagnosis of disease in humans or animals.

Do not use internally or externally in humans or animals.

Storage

Store at 2–8°C. Avoid repeated freeze-thaw cycles. Under these conditions the product is stable for twelve months.

Expiry

See outer packaging.

Safety warnings and precautions

Warning: Contains Sodium Azide in dilute solution. Dispose of waste by flushing with copious amounts of water to avoid the build up of explosive metallic Azides in Copper and Lead plumbing. The total Azide present in each pack is 0.5 mg.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

Component

Biotinylated antibody is supplied in Phosphate Buffered Saline (Sodium Phosphate 0.1 M, NaCl 0.1 M) pH 7.5, containing 1% (w/v) Bovine Serum Albumin and 0.05% (w/v) Sodium Azide.

Description

Purification to ensure species-specificity

The antibody is prepared by hyper-immunizing donkeys with purified immunoglobulin fractions from normal rabbit serum to produce high affinity antibodies. The pooled antiserum is used to produce an immunoglobulin preparation which is then affinity adsorbed to remove cross-reacting antibodies towards rat, human and mouse immunoglobulins. These activities are thoroughly depleted to ensure species-specificity.

Finally, to select for specific binding, the antibodies are purified on affinity columns of rabbit IgG. After washing to remove any remaining non-specific serum components and low affinity antibodies, the rabbit IgG specific antibodies are eluted using carefully selected, mild conditions which minimize aggregation and preserve immunological activity, yet which elute high affinity antibodies.

Preparation of labeled antibody

Biotin is attached to free amino groups on the purified antibodies via a spacer arm. Excess labelling reagent is removed by extensive dialysis.

The degree of biotinylation has been carefully selected to maximize binding to streptavidin, while retaining essentially complete antigen binding ability. Finally, the performance of the biotinylated antibody is evaluated and the concentration is adjusted to ensure that batch to batch variation is minimized and that the product will work in a consistently reproducible manner.

Quality control

The cross-reactivity with other species' IgG and antibody titre is determined in an ELISA system, with antigen bound to the solid phase and using a streptavidin-biotinylated peroxidase complex (RPN1051) to detect bound biotinylated antibody. These tests are carried out on each batch of the biotinylated antibody produced.

Applications

Species-specific antibodies are designed to detect only immunoglobulins from the species against which they are raised. This is desirable for any application where IgG of a second species is present, for example in the immunocytochemical staining of tissue sections and in two-site immunoassays.

Biotinylated antibodies are now used in many ways, including quantitative immunoassay, screening procedures for the detection of hybridoma antibodies, immunocytochemistry and for antigen identification in protein blotting. The biotin-streptavidin system offers flexibility in the choice of detection system in that any of the following may be used- [¹²⁵I]-streptavidin, streptavidin conjugated with fluorochromes or enzymes, streptavidin 'bridge' methods and streptavidin biotinylated enzyme complexes.

The working dilution of the biotinylated antibody will vary according to the application and this reagent has been evaluated for a number of uses. Guidelines for working titres are given below.

1. ELISA

If this reagent is to be used as a link antibody between, for example rabbit IgG and a streptavidin-biotinylated peroxidase complex detection system, we have found in our laboratories that a dilution of 1:40 000 is suitable for the detection of 1 µg of IgG. For greater sensitivity (for example down to 600 pg) the reagent should be diluted rather less (for example 1:5000). Thus 1.0 ml of stock reagent will be sufficient for up to 400 000 wells at the higher dilution if used at 0.1 ml per well in standard microplates. A suitable diluent is Phosphate Buffered Saline containing 0.05% (v/v) Tween™-20.



2. Immunocytochemistry

When using the reagent as a second antibody in immunocytochemistry on sections of formalin-fixed wax-embedded tissue the antibody can be typically diluted 1:200 in Phosphate Buffered Saline. The user may wish to adjust this to obtain the required sensitivity for the tissue under investigation. Assuming that 0.1 ml of the diluted antibody can be used to cover the tissue section then 1.0 ml of stock reagent will be sufficient for up to 2000 slides. If frozen sections are used, acceptable staining may be obtained using even higher dilutions of the reagent.

While it can be expected that the high degree of species specificity will avoid any 'background' staining arising from the biotinylated antibody, its effectiveness is dependent on the binding of the primary antibody specifically to antigen in the tissue section. The biotinylated reagent will assist in the effective demonstration of antigen, but it will not improve the binding performance of the primary antibody.

Antigens which occur infrequently or that have been changed or destroyed by fixing, exposure to organic solvents or to high temperatures would not be expected to localize large amounts of primary antibody and these factors should be considered by the user in making an interpretation of the result.

3. Protein blotting

If this reagent is to be used to localize a detection system to antigen-antibody complex on a nitrocellulose or a nylon blot it would be expected to be sufficient for up to 30 blots of 96 cm², assuming that the membrane is incubated with a volume of 25 ml of biotinylated antibody diluted 1:400.

Related products

Mouse IgG, biotinylated whole antibody (from sheep)	RPN1001
Human IgG, biotinylated whole antibody (from sheep)	RPN1003
Streptavidin alkaline phosphatase conjugate	RPN1234
Streptavidin biotinylated Horseradish Peroxidase complex	RPN1051
Streptavidin Horseradish Peroxidase conjugate	RPN1231
Streptavidin fluorescein	RPN1232
Streptavidin Texas Red™	RPN1233
ECL™ Western blotting detection reagents, 1000 cm ²	RPN2109
ECL Western blotting detection reagents, 2000 cm ²	RPN2209
ECL Western blotting detection reagents, 4000 cm ²	RPN2106
ECL Western blotting detection reagents, 6000 cm ²	RPN2134
ECL Plus Western blotting detection reagents, 1000 cm ²	RPN2132
ECL Plus Western blotting detection reagents, 3000 cm ²	RPN2133
ECL streptavidin-HRP and blocking reagent	RPN2195
ECL blocking agent	RPN2125
Mouse IgG, Horseradish Peroxidase linked whole antibody (from sheep)	NA931
Rabbit IgG, Horseradish Peroxidase linked whole antibody (from donkey)	NA934
Human IgG, Horseradish Peroxidase linked whole antibody (from sheep)	NA933
Mouse IgG, Horseradish Peroxidase linked F(Ab') ₂ fragment (from sheep)	NA9310
Rabbit IgG, Horseradish Peroxidase linked F(Ab') ₂ fragment (from donkey)	NA9340

ECL protein molecular weight markers	RPN2107
Rainbow™ colored protein molecular weight markers (molecular weight range 2350 – 46000)	RPN755E
Rainbow colored protein molecular weight markers (molecular weight range 14 300 – 200 000)	RPN756E
Full Range Rainbow recombinant protein molecular weight markers (molecular weight range 10 000 – 250 000)	RPN800E
Hybond™-ECL nitrocellulose membrane	RPN2020D

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GE Healthcare offices:

GE Healthcare Bio-Sciences AB
Björkgatan 30, 751 84 Uppsala,
Sweden

GE Healthcare Europe GmbH
Munzinger Strasse 5, D-79111 Freiburg,
Germany

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue, P.O. Box 1327,
Piscataway, NJ 08855-1327,
USA

GE Healthcare Bio-Sciences KK
Sanken Bldg. 3-25-1, Hyakunincho,
Shinjuku-ku, Tokyo 169-0073,
Japan

For your local office contact information, visit
www.gelifesciences.com/contact

GE Healthcare UK Limited
Amersham Place
Little Chalfont, Buckinghamshire,
HP7 9NA, UK

<http://www.gelifesciences.com>



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