

Product specification

Anti-sheep/goat Ig, biotinylated whole antibody (from donkey) affinity purified general purpose screening reagent RPN1025

Safety warnings and precautions

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.

We recommend that this product and components are 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. As all chemicals should be considered as potentially hazardous, it is advisable when handling chemical reagents to wear suitable protective clothing, such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In case of contact with skin or eyes, wash immediately with water.

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 1mg.

Purification to ensure specificity

The antibody is prepared by hyper-immunizing donkeys with purified immunoglobulin fractions from normal sheep serum to produce high affinity antibodies.

The pooled antiserum is used to produce an immunoglobulin preparation which is then purified on affinity columns of sheep Ig. After washing to remove any remaining non-specific serum components and low affinity antibodies, the sheep Ig specific antibodies are eluted using carefully selected, mild conditions which minimize aggregation and preserve immunological activity, yet which elute high affinity antibodies. In addition, the anti-sheep Ig antibody has been selected for high binding to goat Ig.

Preparation of labelled 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 whilst retaining essentially complete antigen binding ability. Finally the performance of the biotinylated reagent 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 antibody titre is determined in an ELISA system, with antigen bound to the solid phase and using a streptavidin-biotinylated peroxidase complex (RPN 1051) to detect bound biotinylated antibody. These tests are carried out on each batch of the biotinylated antibody produced.

Formulation

Biotinylated antibody is supplied in phosphate-buffered saline (sodium phosphate 0.1M, NaCl 0.1M) pH7.5, containing 1%(w/v) bovine serum albumin and 0.05%(w/v) sodium azide.

Storage

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

Applications

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.

ELISA

If this reagent is to be used as a link antibody between, for example, a sheep Ig and a streptavidin-biotinylated peroxidase complex detection system, we have found in our laboratories that a dilution of 1:10000 is suitable for the detection of 1µg of Ig. For greater sensitivity (for example down to 600pg) the reagent should be diluted rather less (for example 1:5000). Thus 1.0ml of stock reagent will be sufficient for up to 100000 wells at the higher dilution if used at 0.1ml per well in standard microtitre plates. A suitable diluent is phosphate-buffered saline containing 0.05%(v/v) TweenTM20.

Immunocytochemistry

Whilst potentially applicable to immunocytochemistry this product is not primarily intended for this use. It has not been adsorbed against immunoglobulins of other species to remove cross-reactivities. Therefore, it is likely that if the reagent is used for immunocytochemistry there may be some 'background' staining. The user should consider this in interpreting staining results.

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 96cm², assuming that the membrane is incubated with a volume of 25ml of biotinylated antibody diluted 1:400.

Related products

Mouse Ig, biotinylated whole antibody (from sheep)	RPN 1001
Rat Ig, biotinylated whole antibody (from sheep)	RPN 1002
Human Ig, biotinylated whole antibody (from sheep)	RPN 1003
Rabbit Ig, biotinylated whole antibody (from donkey)	RPN 1004
Streptavidin alkaline phosphatase conjugate	RPN 1234
Streptavidin biotinylated horseradish peroxidase complex	RPN 1051
Streptavidin horseradish peroxidase conjugate	RPN 1231
Streptavidin fluorescein	RPN 1232
Streptavidin Texas Red™	RPN 1233
¹²⁵ I Streptavidin	IM 236
ECL™ Western blotting detection reagents, 1000cm ²	RPN 2109
ECL Western blotting detection reagents, 2000cm ²	RPN 2209
ECL Western blotting detection reagents, 4000cm ²	RPN 2106
ECL Western blotting detection reagents, 6000cm ²	RPN 2134
ECL Plus™ Western blotting detection reagents, 000cm ²	RPN 2132
ECL Plus Western blotting detection reagents, 3000cm ²	RPN 2133
ECL streptavidin-HRP and blocking reagent	RPN 2195
ECL Blocking agent	RPN 2125
Mouse IgG, horseradish peroxidase linked whole antibody (from sheep)	NA 931
Rabbit IgG, horseradish peroxidase linked whole antibody (from donkey)	NA 934
Rat IgG, horseradish peroxidase linked whole antibody (from sheep)	NA 932
Human IgG, horseradish peroxidase linked whole antibody (from sheep)	NA 933
Mouse IgG, horseradish peroxidase linked F(Ab') ₂ fragment (from sheep)	NA 9310
Rabbit IgG, horseradish peroxidase linked F(Ab') ₂ fragment (from donkey)	NA 9340
Rat IgG, horseradish peroxidase linked F(Ab') ₂ fragment (from sheep)	NA 9320
ECL protein molecular weight markers	RPN 2107
Rainbow™ coloured protein molecular weight markers	RPN 755
(molecular weight range 2350 – 46000)	
Rainbow coloured protein molecular weight markers	RPN 756
(molecular weight range 14300 – 200000)	
Full Range Rainbow recombinant protein molecular weight markers	RPN 800
(molecular weight range 10000 – 250000)	
Hybond™ ECL nitrocellulose membrane	RPN 2020D
Hybond PVDF membrane	RPN 2020P
Hyperfilm™ ECL	RPN 2103

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