

Product specification

Streptavidin biotinylated horseradish peroxidase complex RPN 1051

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.

Preparation

Biotin is linked via a spacer arm to horseradish peroxidase under conditions that preserve high enzyme activity and give a high degree of biotinylation as judged by its ability to bind to streptavidin. Excess labelling reagent is removed by gel filtration. Biotinylated enzyme is added to streptavidin to form complexes. The complex is optimized to produce the best possible combination of titre and sensitivity for binding to biotinylated protein. This product is sold on the basis of titre determined in a standard assay. As a result the final protein concentration of the complex (RPN 1051) may vary minimally between batches.

Quality control

For every batch of complex (RPN 1051) that is produced, an ELISA test detecting biotinylated protein immobilized on plastic microtitre plates is used to confirm the titre of the reagent. The substrate used for peroxidase is 2,2-azinobis[3-ethylbenzothiazoline-6-sulphonic acid, diammonium salt](ABTSTM).

Every batch is also QC tested in a Western blotting system. This is performed using HybondTM ECLTM membrane containing tubulin protein and immunodetected with: primary antibody, monoclonal anti-tubulin; secondary antibody RPN 1001, biotinylated anti-mouse Ig; and RPN 1051, streptavidin biotinylated HRP complex. Blots are detected using ECL and ECL PlusTM detection systems.

Formulation

The complex (RPN 1051) is supplied in 2ml of phosphate buffered saline (sodium phosphate 0.1M, sodium chloride 0.1M, pH7.5) containing 1%(w/v) bovine serum albumin and an anti-microbial agent.

Storage and stability

Store at 2-8°C; avoid freezing. Under these conditions the product is stable for 3 months from the date of despatch.

Applications

1) Detection with ECL⁽¹⁾ Western blotting reagents

This reagent has been shown to be suitable for use in ECL, Western blotting applications. The control system used was the detection of monoclonal anti-tubulin. We have found in our laboratories that dilutions of: 1:2000 for monoclonal anti-tubulin; 1:2500 for anti-mouse Ig, biotinylated; and 1:5000 for streptavidin biotinylated HRP complex are suitable for the detection of 5ng of tubulin on Hybond ECL membrane, exposed to HyperfilmTM ECL for 5 minutes.

To achieve the same sensitivity level on Hybond-P PVDF, concentrations would typically be: anti-tubulin 1:3000; RPN 1001 – 1:5000; RPN 1051 – 1:6000.

2) Detection with ECL Plus^(2,3) Western blotting reagents

ECL Plus Western blotting reagents are highly sensitive, giving an increase, for this product, of 4-20 fold over ECL detection.

This property can be utilized in 2 ways:

- 1) Preservation of antibodies that are rare or costly.
- 2) Increase in detectable sensitivity levels.

The control system used was the same as the ECL.

The suitable antibody dilutions, to detect 5ng of tubulin on Hybond ECL membrane, are: anti-tubulin – 1:5000; RPN 1001 – 1:5000; and RPN 1051 1:35000.

For Hybond-P PVDF antibody dilutions are typically: anti-tubulin – 1:10000; RPN 1001 – 1:10000; and RPN 1051 - 1:35000.

Protocol recommendations

Membranes

Nitrocellulose and PVDF membranes are suitable for use with both detection systems. PVDF membrane is highly recommended for use with ECL Plus detection reagents. For high quality results the following guidelines should be followed:

Blocking: Use enough blocking agent to block all non-specific sites. A typical block is 5% non-fat dried milk in PBS-Tween or TBS-Tween eg (RPN 2125). See 'Tech-Tips'. No. 136 available from Amersham Biosciences, for further details.

Washing: The volume of wash buffer (eg PBS-T or TBS-T) must be sufficient to cover the membrane completely.

Determination of optimum antibody concentrations

ECL detection

ECL Western blotting is a very sensitive technique. As such it is essential to optimize the system under study for high signal and low background for both primary and secondary antibodies and streptavidin biotinylated HRP complex.

Dot blots are a quick and effective method of determining the optimum dilutions required for primary and secondary antibodies. Optimization details are set out in the RPN 2106/2108/2109/2209/2134 booklets and 'Tech-Tips' No 129 available from Amersham Biosciences. These methods can be extended to incorporate optimization of streptavidin biotinylated HRP complex (RPN 1051).

ECL Plus detection

Due to the improved sensitivity of ECL Plus compared to ECL, optimization details as set out in the RPN 2132/2133 booklets and 'Tech-Tips' No. 169 available from Amersham Biosciences are recommended.

Typical streptavidin biotinylated HRP complex dilution ranges

ECL for nitrocellulose membrane 1:1000 to 1:5000

ECL Plus for nitrocellulose membrane 1:2000 to 1:10000

For PVDF membrane the use of higher dilutions may be necessary.

The exact concentration of the complex will always be dependent upon the primary and secondary antibodies used and the sensitivity and exposure times required.

Detection

Ensure any excess ECL or ECL Plus detection reagents are sufficiently drained prior to exposure.

Exposure times

ECL – exposure times of 1 to 15 minutes are suggested.

ECL Plus – initial exposure times of 1 to 5 minutes are suggested.

Signal can still be obtained up to 24 hours after the application of ECL Plus reagents, and for this exposure times of 1 to 2 hours may be required.

ELISA

If this reagent is to be used to detect biotinylated immunoglobulin we have found in our laboratories that a dilution of 1:16000 is suitable for the detection of 1µg of 1g. For greater sensitivity, the reagent should be diluted rather less (for example 1:1600). Thus 1.0ml of stock reagent will be sufficient for up to 160000 wells at the higher dilution if used at 0.1ml per well in standard microtitre plates. A suitable diluent is phosphate-buffered saline containing 0.25%(w/v) gelatin. It has been found in a separate ELISA test that a 1:1000 dilution of the complex will detect less than 1 nanogram of the biotinylated protein.

Immunocytochemistry

When using the complex (RPN 1051) for detection in immunocytochemistry on sections of formalin-fixed wax-embedded tissue, the reagent can be typically diluted 1:100 in phosphate-buffered saline. The user may wish to adjust the required sensitivity for the antigen under investigation. Assuming that 0.1ml of the diluted complex (RPN 1051) can be used to cover the tissue section, then 1.0ml of stock reagent will be sufficient for up to 600 slides. If frozen sections or 'fresh' cell preparations are used, acceptable staining may be obtained using even higher dilutions of the reagent.

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 horseradish peroxidase conjugate	RPN 1231
Streptavidin fluorescein	RPN 1232
Streptavidin Texas Red™	RPN 1233
ECL Western blotting detection reagents	RPN 2109/2209/2106/2134
ECL Plus Western blotting detection reagents	RPN 2132/2133
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
Full Range Rainbow™ recombinant protein molecular weight markers (molecular weight range 10000 – 250000)	RPN 800
Hybond ECL nitrocellulose membrane	RPN 2020D
Hybond PVDF membrane	RPN 2020P
Hyperfilm ECL	RPN 2103

References

- 1) WHITEHEAD, T.P. *et al.*, *Clin Chem.*, **25**, pp.1531-1546, 1979.
- 2) AKHAVEN-TAFTI, H. *et al.*, *Clin. Chem.*, **41**, pp.1368-1369, 1995.
- 3) AKHAVEN-TAFTI, H. *et al.*, *Biolum and Chemilum. Fundamentals and Applied Aspects*, pp. 199-202, Chichester, 1994.

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<http://www.amershambiosciences.com>

Amersham Biosciences UK Limited Amersham Place

Little Chalfont Buckinghamshire England HP7 9NA

Amersham Biosciences AB SE-751 84 Uppsala Sweden

Amersham Biosciences Inc 800 Centennial Avenue PO Box 1327 Piscataway NJ08855 USA

Amersham Biosciences Europe GmbH Munzinger Strasse 9 D-79111 Freiburg Germany

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