

Amersham Streptavidin-Biotinylated Horseradish Peroxidase Complex

Product Specification Sheet

Code: RPN1051

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.

Handling

Storage

Store at 2–8°C, avoid freezing. Under these conditions the product is stable until expiry date.

Expiry

See outer packaging.

Safety warnings and precautions

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.

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 (RPN1051) may vary minimally between batches.

Component

The complex (RPN1051) is supplied in 2 ml of Phosphate Buffered Saline (Sodium Phosphate 0.1 M, Sodium Chloride 0.1 M, pH 7.5) containing 1% (w/v) Bovine Serum Albumin and an anti-microbial agent.

Quality control

For every batch of complex (RPN1051) that is produced, an ELISA test detecting biotinylated protein immobilized on plastic microplates 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](ABTS™).

Every batch is also QC tested in a Western blotting system. This is performed using Hybond™ ECL™ membrane containing Tubulin protein and

immunodetected with: primary antibody, monoclonal anti-tubulin; secondary antibody RPN1001, biotinylated anti-mouse Ig; and RPN1051, Streptavidin biotinylated HRP complex. Blots are detected using ECL and ECL Plus™ detection systems.

Applications

1. Protein blotting

a) 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 5 ng of tubulin on Hybond ECL membrane, exposed to Hyperfilm™ ECL for 5 minutes.

To achieve the same sensitivity level on Hybond-P PVDF, concentrations would typically be: anti-tubulin 1:3000; RPN1001 - 1:5000; RPN1051 - 1:6000.

b) 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:

- Preservation of antibodies that are rare or costly
- Increase in detectable sensitivity levels

The control system used was the same as for ECL.

The suitable antibody dilutions, to detect 5 ng of Tubulin on Hybond ECL membrane are: anti-tubulin - 1:5000; RPN1001 - 1:5000; and RPN1051 1:35 000. For Hybond-P PVDF, dilutions are typically: anti-tubulin - 1:10 000; RPN1001 - 1:10 000; and RPN1051 - 1:35 000.

2. ELISA

If this reagent is to be used to detect biotinylated immunoglobulin we have found in our laboratories that a dilution of 1:16 000 is suitable for the detection of 1 µg of Ig. For greater sensitivity, the reagent should be diluted rather less (for example 1:1600). Thus 1.0 ml of stock reagent will be sufficient for up to 160 000 wells at the higher dilution if used at 0.1 ml per well in standard microplates. A suitable diluent is Phosphate-Buffered Saline containing 0.25% (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.

3. Immunocytochemistry

When using the complex (RPN1051) 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 0.1 ml of the diluted complex (RPN1051) can be used to cover the tissue section, then 1.0 ml 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.

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 RPN2125). See 'Tech-Tips' No. 136 available from GE Healthcare, 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 RPN2106/2108/2109/2209/2134 booklets and 'Tech-Tips' No. 129 available from GE Healthcare. These methods can be extended to incorporate optimization of Streptavidin biotinylated HRP complex (RPN1051).

ECL Plus detection

Due to the improved sensitivity of ECL Plus compared to ECL, optimization details as set out in the RPN2132/2133 booklets and 'Tech-Tips' No. 169 available from GE Healthcare are recommended.

Typical Streptavidin biotinylated HRP complex dilution ranges:

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|--------------------------------------|--------------------|
| ECL for Nitrocellulose membrane | 1:1000 to 1:5000 |
| ECL Plus for Nitrocellulose membrane | 1:2000 to 1:10 000 |

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.

Related products

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| Mouse Ig, biotinylated whole antibody (from sheep) | RPN1001 |
| Human Ig, biotinylated whole antibody (from sheep) | RPN1003 |
| Rabbit Ig, biotinylated whole antibody (from donkey) | RPN1004 |
| Streptavidin Alkaline Phosphatase conjugate | RPN1234 |
| Streptavidin Horseradish Peroxidase conjugate | RPN1231 |
| Streptavidin Fluorescein | RPN1232 |
| Streptavidin Texas Red™ | RPN1233 |
| ECL Western blotting detection reagents | RPN2109/2209/2106/2134 |
| ECL Plus Western blotting detection reagents | RPN2132/2133 |
| ECL blocking agent | RPN2125 |
| Mouse IgG, Horseradish Peroxidase linked whole antibody (from sheep) | NA931 |
| Rabbit IgG, Horseradish Peroxidase linked whole antibody (from donkey) | NA934 |
| Rat IgG, Horseradish Peroxidase linked whole antibody (from sheep) | NA932 |
| Human IgG, Horseradish Peroxidase linked whole antibody (from sheep) | NA933 |
| Mouse IgG, Horseradish Peroxidase linked F(Ab) ₂ fragment (from sheep) | NA9310 |

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| Rabbit IgG, Horseradish Peroxidase linked F(Ab) ₂ fragment (from donkey) | NA9340 |
| Rat IgG, Horseradish Peroxidase linked F(Ab) ₂ fragment (from sheep) | NA9320 |
| ECL protein molecular weight markers | RPN2107 |
| Full Range Rainbow™ recombinant protein molecular weight markers (molecular weight range 10 000 - 250 000) | RPN800 |
| Hybond™-ECL Nitrocellulose membrane | RPN2020D |
| Hybond PVDF membrane | RPN2020P |
| Hyperfilm™ ECL | RPN2103 |

References

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

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