Product specification

Streptavidin-horseradish peroxidase conjugate RPN 1231

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

The conjugation of horseradish peroxidase and streptavidin is achieved using the periodate coupling method described by Nakane and co-workers[(1)].

This product is sold on the basis of titre in an ELISA assay and performance in a Western blot. As a result the final protein concentration of the conjugate (RPN 1231) will vary only minimally between batches.

Quality control

For every batch of peroxidase labelled streptavidin (RPN 1231) that is produced, an ELISA is used to determine the titre of the reagent. The substrate used for the peroxidase is 2,2′-azino-di-[3-ethylbenzthiazoline sulphonate, 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 RPN 1001, anti-mouse IgG biotinylated; and RPN 1231, streptavidin HRP conjugate. Blots are detected using ECL and ECL Plus™ detection systems.

Formulation

The conjugate (RPN 1231) is supplied in 2ml of phosphate buffered saline (sodium phosphate 0.1M, sodium chloride 0.1M, pH7.4) containing 1%(w/v) bovine serum albumin and an antimicrobial agent.

Amersham Biosciences

Storage
Store at 2-8°C; avoid freezing. Under these conditions the product is stable for at least 12 months from the date of despatch.

Applications
1) Detection with ECL[2] 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 IgG, biotinylated; and 1:35000 for streptavidin HRP conjugate are suitable for the detection of 5ng 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; RPN 1001 – 1:5000; RPN 1231 – 1:40000.

2) Detection with ECL Plus[3,4] 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 for 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 1231 1:85000.
For Hybond-P PVDF antibody dilutions are typically: anti-tubulin – 1:10000; RPN 1001 – 1:10000; and RPN 1231 1:85000.

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 (RPN 2125) in PBS-Tween™ or TBS-Tween. 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 HRP conjugate.
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 utilized for optimization of streptavidin HRP conjugate.

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 HRP conjugate 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 conjugate 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 of 2 hours may be required.

ELISA

If this reagent is to be used to detect biotinylated immunoglobulins, we have found in our laboratories that a dilution of 1:5000 is suitable for the detection of 1µg of Ig. For greater sensitivity (for example down to 80pg), the reagent should be diluted rather less (for example 1:500). Thus 1.0ml of stock reagent will be sufficient for up to 50000 wells at the higher dilution if used at 1.0ml per well in standard microtitre plates. A suitable diluent is phosphate-buffered saline containing 0.1%(v/v) Tween 20.

Immunocytochemistry

When using the conjugate (RPN 1231) as a detection layer in immunocytochemistry on sections of formalin-fixed wax-embedded tissue, the reagent can be typically diluted 1:300 in phosphate buffered saline. The user may wish to adjust this to obtain the required sensitivity for the tissue under investigation. Assuming that 0.1ml of the diluted conjugate can be used to cover the tissue section, then 1.0ml of stock reagent will be sufficient for up to 3000 slides. If frozen sections are used, acceptable staining may be obtained using higher dilutions of the reagent.

Related products

ECL Western blotting detection reagents	RPN 2106/2108/2109/2209/2134
ECL Plus Western blotting detection reagents	RPN 2132/2133
Anti-mouse IgG, biotinylated	RPN 1001
Anti-rabbit IgG, biotinylated	RPN 1004
Hybond ECL membrane	RPN 2020D
Hybond-P PVDF membrane	RPN 2020F
Hyperfilm ECL	RPN 2103/2104/1681/1674
ECL protein molecular weight markers	RPN 2107
ECL blocking agent	RPN 2125

References

1) WILSON, M.B. and NAKANE, P.K., pp.215-244 in *Immunofluorescence and Related Staining Techniques*, edited by Knapp W. *et al.,* Elsevier North Holland, 1978.
2) WHITEHEAD, T.P. *et al., Clin. Chem.*, **25**, pp.1531-1546, 1979.
3) AKHAVEN-TAFTI, H. *et al., Clin. Chem.*, **41**, pp.1368-1369, 1995.
4) AKHAVEN-TAFTI, H. *et al., Biolum. and Chemilum. Fundamentals and Applied Aspects* pp.199-202, Chichester. 1994.

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

RPN1231PS/AD