ECL™

Product specification

Anti-mouse IgG, peroxidase-linked whole antibody (from sheep) affinity purified general purpose reagent NXA 931

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 the case of contact with skin or eyes, wash immediately with water.

Purification to ensure specificity

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

The pooled antiserum is used to produce an immunoglobulin preparation which is then purified on affinity columns of mouse IgG. After washing to remove any remaining non-specific serum components and low affinity antibodies, the specific antibodies are eluted using carefully selected, mild conditions which minimize aggregation and preserve immunological activity, yet which elute high affinity antibodies.

Preparation of labelled antibody

The enzyme horseradish peroxidase is attached to the immunoglobulin molecules using an adaptation of the periodate oxidation technique[1]. This method has been found not to affect the effective binding of the antibody to the antigen or the activity of the enzyme.

Quality control

For every batch of enzyme-linked antibody that is produced the antibody titre is determined in an ELISA. The substrate used for the peroxidase is 2,2'-azinobis[3-ethylbenzothiazoline sulphonate, diammonium salt], ABTS™.

Amersham Biosciences

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; and secondary antibody NXA 931, anti-mouse IgG, HRP GPR. Blots are detected using ECL and ECL Plus™ detection systems.

Formulation

Horseradish peroxidase conjugated antibody is supplied in phosphate-buffered saline (sodium phosphate 0.1M, NaCl 0.1M) pH7.5, containing 1%(w/v) bovine serum albumin and an anti-microbial agent.

Storage

Store at 2-8°C; avoid freezing. Avoid repeated freeze-thaw cycles. Under these conditions the product is stable for twelve months from the date of despatch.

Applications

Protein blotting

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; and 1:5000 for anti-mouse IgG, HRP GPR are suitable for the detection of 3ng 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 - 1:2000; and NXA 931 - 1:10000.

2) Detection with ECL Plus[3,4] Western blotting reagent

ECL Plus Western blotting reagent is highly sensitive, giving an increase, for this antibody, of 4 to 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

Use the same control system as for ECL.
The suitable antibody dilutions, to detect 3ng of tubulin on Hybond ECL membrane are: anti-tubulin - 1:5000; and NXA 931 - 1:25000.
For Hybond-P PVDF antibody dilutions are typically: anti-tubulin - 1:10000; and NXA 931 - 1:50000.

3) Colorimetric detection

A dilution of 1:300 is recommended.

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. 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.

Optimization of primary and secondary antibodies

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.

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.

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 anti-mouse secondary antibody 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 secondary antibody will always be dependant upon the primary antibody 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 to 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 mouse immunoglobulins, we have found in our laboratories that a dilution of 1:6000 is suitable for the detection of 1µg of IgG. For greater sensitivity (for example down to 300pg) the reagent should be diluted rather less (for example 1:500). Thus 1.0ml of stock reagent will be sufficient for up to 60000 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) Tween 20.

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. More suitable reagents for immunocytochemistry are peroxidase labelled anti-mouse IgG, whole antibody (NA 931) or peroxidase labelled anti-mouse IgG, $F(ab')_2$ fragments (NA 9310), both of which are species-specific.

Related products

ECL Western blotting detection reagents	**RPN 2106/2108/2109/2209/2134**
ECL Plus Western blotting detection system	**RPN 2132/2133**
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**

References

1) NAKANE, P.K. and KAWAOI, A., *Journal of Histochemistry and Cytochemistry*, **22**, pp. 1084-1091, 1974.
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.

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