ECL™

Product specification

Anti-human IgG, peroxidase-linked species-specific whole antibody (from sheep) NA 933

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 species-specificity

The antibody is prepared by hyper-immunising sheep with purified immunoglobulin fractions from normal human serum to produce high affinity antibodies. The pooled antiserum is used to produce an immunoglobulin preparation which is then affinity adsorbed to remove cross-reacting antibodies towards rat, mouse and rabbit immunoglobulins. These activities are thoroughly depleted to ensure species-specificity.

Finally, to select for specific binding to human IgG, the antibodies are purified using an affinity column of human IgG. After washing to remove non-specific serum components and low affinity antibodies, the species-specific antibodies are eluted using carefully selected, mild conditions which minimise aggregation and preserve immunological activity, yet which will 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.

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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™.
Every batch is also QC tested in a dot blotting system. This is performed using Hybond™ ECL™ membrane containing human IgG protein, immunodetected with secondary antibody, Anti-human IgG, HRP (NA 933). 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. Under these conditions the product is stable for 12 months from the date of despatch.

Applications
Protein blotting
1) Detection with ECL[2] Western blotting reagents
The control system used was the detection of human IgG.
We have found in our laboratories that a dilution of 1:1000 for anti-human IgG, HRP is suitable for the detection of 2.5ng of human IgG dotted on to Hybond ECL membrane and exposed to Hyperfilm™ ECL for 5 minutes.
To achieve the same sensitivity level on Hybond-P PVDF, the concentration would typically be 1:2000.

2) Detection with ECL Plus[3,4] Western blotting reagents
ECL Plus reagent is highly sensitive, giving an increase, for this antibody, of 4-20 fold over ECL detection.
This property can be utilised 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 dilution, to detect 2.5ng of human IgG dotted on to Hybond ECL membrane is NA 933 - 1:10000.
For Hybond-P PVDF, the antibody concentration would typically be 1:20000.

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.

Optimisation of primary and secondary antibodies

ECL detection

ECL Western blotting is a very sensitive technique. As such it is essential to optimise 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. Optimisation details are set out in the RPN 2106/2108/2109/2209/2134 booklet and 'Tech-Tips' No.129 available from Amersham Biosciences .

ECL Plus detection

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

Typical anti-human 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 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 human 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

The use of this anti-human reagent for immunocytochemistry may be different from the use of other anti-species antibodies. Usually such anti-species antibodies are used to recognise primary antibodies, for example, from rats, rabbits or mice. However, human antibodies as primary reagents are not widely available, but there are applications such as the study of auto-antibody production in which this anti-human reagent may be used. It must be accepted that background staining will arise as a result of the antibody recognising ubiquitous IgG in human tissue sections. This must be considered by the user during interpretation of the results, and the reagent should be titrated to give a 'signal-to noise' ratio that is considered acceptable. Suitable controls should be included.

Related products

ECL Western blotting detection reagents	**RPN 2106/2108/2109/2209/2134**
ECL Plus Western blotting detection systems	**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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