product code

NA9310

ECL Anti-mouse IgG, peroxidase-linked species-specific F(ab')$_2$ fragment (from sheep)

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.

Description

Purification to ensure species-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 affinity adsorbed to remove cross-reacting antibodies towards rat, human and rabbit immunoglobulins. These activities are thoroughly depleted to ensure species-specificity.

Finally, to select for specific binding to mouse IgG, the antibodies are purified using an affinity column of mouse 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 minimize aggregation and preserve immunological activity, yet which will elute high affinity antibodies.

The F(ab')$_2$ fragments are produced by digestion of the whole antibodies with pepsin. Undigested IgG Fc fragments and pepsin are removed by gel filtration. The purity of the separated F(ab')$_2$ fragments is checked by gel electrophoresis.

Preparation of labelled antibody

The enzyme Horseradish Peroxidase is attached to the F(ab')$_2$ fragments 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™.

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 NA9310, anti-mouse IgG, HRP F(ab')$_2$ fragment. Blots are detected using ECL and ECL Plus™ detection systems.

Handling

Storage
Store at 2-8 °C. Do not freeze. Under these conditions, the product is stable for at least 12 months from the date of despatch.

Expiry
See outer packaging.

Component
Horseradish Peroxidase conjugated F(ab')$_2$ fragments are supplied in Phosphate Buffered Saline (Sodium Phosphate 0.1 M, NaCl 0.1 M) pH7.5, containing 1%(w/v) Bovine Serum Albumin and an anti-microbial agent.

Safety warnings and precautions

Warning: For research use only.
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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.

Applications

1 Protein blotting

a) 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 F(ab')$_2$ fragments are suitable for the detection of 3 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 - 1:2000; and NA9310 - 1:10000.

b) Detection with ECL Plus(3,4) Western blotting reagents

ECL Plus Western blotting reagent is highly sensitive, giving an increase, for this antibody, 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 3 ng of tubulin on Hybond ECL membrane are: anti-tubulin - 1:5000; and NA9310 - 1:25000.

For Hybond-P PVDF antibody dilutions are typically: anti-tubulin - 1:10000; and NA9310-1:50000.

c) Colorimetric detection

A dilution of 1:300 is recommended.

❷ ELISA

If this reagent is to be used to detect mouse immunoglobulins, we have found in our laboratories that a dilution of 1:9000 is suitable for the detection of 1 µg of IgG. For greater sensitivity (for example down to 300 pg) the reagent should be diluted rather less (for example 1:1000). Thus 1.0 ml of stock reagent will be sufficient for up to 90000 wells at the higher dilution if used at 0.1 ml per well in standard microtitre plates. A suitable diluent is Phosphate-Buffered Saline containing 0.05%(v/v) Tween™ 20.

❸ Immunocytochemistry

When using the reagent as a second antibody in immunocytochemistry on sections of formalin-fixed wax-embedded tissue the antibody can be typically diluted 1:100 in Phosphate-Buffered Saline. The user may wish to adjust this to obtain the required sensitivity for the tissue under investigation. Assuming that 0.1 ml of the diluted antibody can be used to cover the tissue section then 1.0 ml of stock reagent will be sufficient for up to 1000 slides. If frozen sections 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. 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 RPN2106/2108/2109/2209/2134 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 RPN2132/2133 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 dependent 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 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

ECL Western blotting detection reagents	RPN2106/2108/2109/2209/2134
ECL Plus Western blotting detection system	RPN2132/2133
Hybond ECL membrane	RPN2020D
Hybond-P PVDF membrane	RPN2020F
Hyperfilm ECL	RPN2103/2104/1681/1674
ECL protein molecular weight markers	RPN2107

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.

Legal

Product information

Product name	code
ECL Anti-mouse IgG, peroxidase-linked species-specific $F(ab')_2$ fragment (from sheep)	NA9310

Related products

See previous list

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