ECL

Product specification

Anti-rat IgG, peroxidase-linked species-specific F(ab′)$_2$ fragment (from goat)
NA 9350

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 the 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-immunizing goats with purified immunoglobulin fractions from normal rat 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 mouse, human and rabbit immunoglobulin. These activities are thoroughly depleted to ensure species-specificity.

Finally, to select for specific binding to rat IgG, the antibodies are purified using an affinity column of rat 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.

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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 Western blotting system. This is performed using Hybond™ ECL™ membrane containing recombinant murine interferon-gamma protein and immunodetected with primary antibody rat (monoclonal) anti-mouse interferon-gamma (BIOSOURCE AMC4834) and secondary antibody anti-rat IgG, HRP $F(ab')_2$ fragment (NA 9350). Blots are detected using ECL and ECL Plus™ detection systems.

Formulation
Horseradish peroxidase conjugated $F(ab')_2$ fragments are 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 a minimum of 3 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 rat (monoclonal) anti-mouse IFN-gamma (AMC4834).
We have found in our laboratories that dilutions of 1:2000 for AMC4834 and of 1:3000 for NA 9350 are suitable for the detection of 6.25ng of murine IFN-gamma on Hybond ECL and Hybond-P PVDF membranes, exposed to Hyperfilm™ ECL for 5 minutes.

2) 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 to 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 6.25ng of murine IFN-gamma on Hybond ECL membrane are AMC4834 - 1:2500 and NA 9350 - 1:10000.
For Hybond-P PVDF antibody dilutions are typically AMC4834 - 1:5000 and NA 9350 - 1:10000.

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 observed:
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.

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-rat 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 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 rat immunoglobulins, we have found in our laboratories that a dilution of 1:9000 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 1:5000). Thus 1.0ml of stock reagent will be sufficient for up to 90000 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

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 0.1ml of the diluted antibody can be used to cover the tissue section then 1.0ml 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.

Related products

ECL Western blotting detection reagents	**RPN 2106/2108/2109/2209/2134**
ECL Plus Western blotting detection system	**RPN 2132/2133**
ECL Plus Western blotting reagent pack	**RPN 2124**
ECL blocking agent	**RPN 2125**
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**
Rainbow™ coloured protein molecular weight markers	**RPN 755/756/800**

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.192-202, Chichester, 1994.

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