Amersham™ ECL™ Anti-rat IgG, Horseradish Peroxidase-Linked Species-Specific Whole Antibody (from goat)

Product Specification Sheet

Code: NA935

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.

Storage
Store at 2–8°C. Do not freeze. Under these conditions, the product is stable for at least 3 months from the date of despatch.

Expiry
See outer packaging.

Safety warnings and precautions
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.

Component
Horseradish Peroxidase conjugated antibody is supplied in Phosphate Buffered Saline (Sodium Phosphate 0.1 M, NaCl 0.1 M) pH 7.5, containing 1% (w/v) Bovine Serum Albumin and an anti-microbial agent.

Description
Purification to ensure species-specificity
The antibody is prepared by hyper-immunizing goats with purified immunoglobulin fractions from normal rabbit 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 immunoglobulins. 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.

Preparation of labeled 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™.

Every batch is also QC tested in a Western blotting system. This is performed using Amersham Protran Premium membrane containing recombinant murine IFN-gamma protein, immunodetected with primary antibody rat (monoclonal) anti-mouse IFN-gamma (INVITROGEN) and secondary antibody anti-rat IgG, HRP (NA935). Blots are detected using Amersham ECL and Amersham ECL prime™ detection systems.

Applications
Protein blotting
a) Detection with Amersham ECL (2) Western blotting reagents
This reagent has been shown to be suitable for use in Amersham 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 NA935 are suitable for the detection of 50 ng of murine IFN-gamma on Amersham Protran Premium membranes, exposed to Amersham Hyperfilm™ ECL for 5 minutes.

To achieve the same sensitivity level on Amersham Hybond™-P PVDF, concentrations would typically be AMC4834 - 1:5000 and NA935 - 1:10 000.

b) Detection with Amersham ECL Prime (3, 4) Western blotting reagents
Amersham ECL Prime Western blotting reagent is highly sensitive, giving an increase, for this antibody, of 4 to 20 fold over Amersham 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 Amersham ECL.

The suitable antibody dilutions, to detect 50 ng of murine IFN-gamma on Amersham Protran Premium membrane are AMC4834 - 1:2500 and NA935 - 1:10 000. For Amersham Hybond-P PVDF, antibody dilutions are typically AMC4834 - 1:5000 and NA935 - 1:10 000.

c) Colorimetric detection
A dilution of 1:300 is recommended.

ELISA
If this reagent is to be used to detect rat immunoglobulins, we have found in our laboratories that a dilution of 1:6000 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 60 000 wells at the higher dilution if used at 0.1 ml per well in standard microplates. 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 0.1 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 Amersham ECL Prime 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 5% non-fat dried milk (RPN2125) in PBS Tween or TBS Tween. See 'Tech-Tips' No. 136 available from GE 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

Amersham ECL detection

Enhanced Chemiluminecence Western blotting detection 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 for Amersham ECL are set out in the RPN2106/2108/2109/2209/2134 booklets and 'Tech-Tips' No. 129 available from GE.

Amersham ECL Prime detection

Due to the improved sensitivity of Amersham ECL Prime compared to Amersham ECL, optimization details as set out in the RPN2132/2133 booklets and 'Tech-Tips' No. 169 available from GE are recommended.

Typical anti-rat secondary antibody dilution ranges:

Amersham ECL for nitrocellulose membrane 1:1000 to 1:5000

Amersham ECL Prime for nitrocellulose membrane 1:12 000 to 1:10 000

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 Amersham ECL or Amersham ECL Prime detection reagents are sufficiently drained prior to exposure.

Exposure times:
Amersham ECL - exposure times of 1 to 5 minutes are suggested.

Amersham ECL Prime - initial exposure times of 1 to 5 minutes are suggested

Signal can still be obtained up to 24 hours after the application of Amersham ECL Prime reagents, and for this exposure times of 1 to 2 hours may be required.

imagination at work

Related products

Amersham ECL Western blotting detection reagents	RPN2106/2109/ 2209/2134
Amersham ECL Prime Western blotting detection system	RPN2232/2236
Amersham ECL Western blotting reagent pack	RPN2124
Amersham ECL blocking agent	RPN2125
Amersham Protran Premium	10600003
Amersham Hybond-P PVDF membrane	10600023
Amersham Hyperfilm ECL	28906835/28906836/ 28906837/28906838/ 28906839
Amersham ECL Rainbow™ Molecular Weight Markers	RPN755E/756E/800E

References

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2. Whitehead, T.P. *et al., Clin. Chem.,* **25**, 1531-1546 (1979).

3. Akhaven-Tafti, H. *et al., Clin. Chem.,* **45**, 1368-1369 (1995).

4. Akhaven-Tafti, H. *et al., Biolum. And Chemilum. Fundamentals and Applied Aspects,* 199-202, Chichester (1994).

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