Amersham ECL

Anti-rabbit IgG, Horseradish Peroxidase-Linked Species-Specific Whole Antibody (from donkey) Product Specification Sheet

Introduction

Important

Read these instructions carefully before using the products.

Intended use

The products are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

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.

For use and handling of the products in a safe way, refer to the Safety Data Sheets.

Storage

Store at 2–8°C. Do not freeze. Under these conditions, the product is stable for at least 6 months from the date of despatch.

Expiry

See outer packaging.

Components

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 donkeys 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 rat, human and mouse immunoglobulins. These activities are thoroughly depleted to ensure species-specificity.

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

Every batch is also QC tested in a Western blotting system. This is performed using Amersham™ Protran™ Premium membrane containing serially diluted beta-galactosidase protein and immunodetected with anti-beta-galactosidase primary antibody and anti-rabbit IgG HRP secondary antibody. Blots are detected using ECL™ and ECL Prime detection systems.

Applications

Protein blotting

1. **Detection with Amersham ECL Western blotting reagents (2)**

   This reagent has been shown to be suitable for use in Amersham ECL Western blotting applications. The control system used was the detection of anti-beta galactosidase antibody.

   We have found in our laboratories that dilutions of 1:5000 for anti-Beta Galactosidase and 1:50 000 for anti-rabbit IgG, HRP secondary antibody are suitable for the detection of 6 ng of betagalactosidase on Amersham Protran Premium membrane, exposed to Amersham Hyperfilm™ ECL for 5 minutes.

   To achieve the same sensitivity level on Amersham Hybond™, concentrations would typically be anti-beta galactosidase - 1:5000 and NA934 - 1:100 000.

2. **Detection with Amersham ECL Prime Western blotting reagents (3, 4)**

   Amersham ECL Prime 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

The control system used was the same as for ECL.

The suitable antibody dilutions, to detect 6 ng of beta-galactosidase on Amersham Protran Premium membrane are anti-beta galactosidase - 1:10 000 and NA934 - 1:100 000. For Amersham Hybond membrane antibody dilutions are typically anti-beta galactosidase - 1:20 000 and NA934 - 1:200 000.

3. **Colorimetric detection**

   A dilution of 1:300 is recommended.

ELISA

If this reagent is to be used to detect rabbit 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 1:5000). 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 typically be diluted 1:100 in Phosphate-Buffered Saline. The user may wish to adjust this to obtain the required sensitivity for the tissue under investigation. 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 in PBS Tween or TBS Tween.
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

Amersham 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*.

Amersham ECL Prime detection

Due to the improved sensitivity of Amersham ECL Prime compared to ECL, optimization details as set out in the *RPN2132/2133 booklets*.

Typical anti-mouse secondary antibody dilution ranges:

Amersham ECL for nitrocellulose membrane 1:5000 to 1:50 000
Amersham ECL Prime for nitrocellulose membrane 1:10 000 to 1:100 000.

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 15 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.

Related products

Amersham ECL Western blotting detection reagents	RPN2106/2108/2109/2209/2134
Amersham ECL Prime Western blotting detection system	RPN2232 & RPN2236
Amersham Protran Premium	10600048
Amersham Hybond membrane	10600058
Amersham Hyperfilm ECL film	28906835/28906836/ 28906837/28906838/ 28906839
Amersham ECL Blocking Agent	RPN2125
Amersham Rainbow™ Molecular Weight Markers	RPN800E/RPN756E/RPN755E

References

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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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