Amersham ECL

Anti-Human IgG, Horseradish Peroxidase-Linked Species-Specific Whole Antibody (from sheep) 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.

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

Safety

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.

Description

Purification to ensure species-specificity

The antibody is prepared by hyper-immunizing 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 speciesspecific 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 Hybond™ ECL™ membrane containing human IgG protein, immunodetected with secondary antibody, Anti-human IgG, HRP (NA933). Blots are detected using ECL and ECL Plus detection systems.

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 2.5 ng 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 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 dilution, to detect 2.5 ng of human IgG dotted on to Hybond ECL membrane is NA933 - 1:10000.

   For Hybond-P PVDF, the antibody concentration would typically be 1:20000

3. **Colorimetric detection**

   A dilution of 1:300 is recommended.

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 1 µg of IgG. For greater sensitivity (for example down to 300 pg) the reagent should be diluted rather less (for example 1:500). Thus 1.0 ml of stock reagent will be sufficient for up to 60000 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.

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.

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 5% non-fat dried milk in PBS Tween or TBS Tween.

See *'Tech-Tips' No. 136* available from Cytiva, 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 Cytiva.

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 Cytiva 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 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**, 1084-1091 (1974).
2. Whitehead, T.P. et al., *Clin. Chem*., **25**, 1531-1546 (1979).
3. Akhaven-Tafti, H. et al., *Clin. Chem*., **41**, 1368-1369 (1995).
4. Akhaven-Tafti, H. *et al., Biolum. And Chemilum. Fundamentals and Applied Aspects*, 199-202, Chichester, (1994).

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