



Product specification

Anti-rabbit IgG, peroxidase-linked species-specific F(ab')₂ fragment (from donkey) NA 9340

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 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-immunising 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 minimise 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 and 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 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], ABTSTM. Every batch is also QC tested in a Western blotting system. This is performed using HybondTM ECLTM membrane containing beta-galactosidase protein and immunodetected with primary antibody anti-beta galactosidase (Cappel) and secondary antibody NA 9340, anti-rabbit IgG, HRP F(ab')₂ fragment. Blots are detected using ECL and ECL PlusTM detection system.

Formulation

Horseradish peroxidase 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 12 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 anti-beta galactosidase.

We have found in our laboratories that dilutions of 1:5000 for Anti-beta galactosidase and 1:50000 for NA 9340 are suitable for the detection of 6ng of beta-galactosidase on Hybond ECL membrane, exposed to HyperfilmTM ECL for 5 minutes. To achieve the same sensitivity level on Hybond-P PVDF, dilutions would typically be Anti-beta galactosidase - 1:5000 and NA 9340 - 1:100000.

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 utilised 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 6ng of beta-galactosidase on Hybond ECL membrane are anti-beta galactosidase - 1:10000 and NA 9340 - 1:100000

For Hybond-P PVDF, dilutions would typically be Anti-beta galactosidase - 1:20000 and NA 9340 - 1:200000.

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 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.

Optimisation of primary and secondary antibodies:

ECL detection

ECL Western blotting is a very sensitive technique. As such it is essential to optimise 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. Optimisation details are set out in the RPN

2106/2108/2109/2209/2134 booklet and 'Tech-Tips' No. 129 available from Amersham Biosciences.

ECL Plus detection

Due to the improved sensitivity of ECL Plus compared to ECL, optimisation details as set out in the RPN 2132/2133 booklet and 'Tech-Tips' No. 169 available from Amersham Biosciences are recommended.

Typical anti-rabbit secondary antibody dilution ranges:

ECL for nitrocellulose membrane 1:5000 to 1:50000

ECL Plus for nitrocellulose membrane 1:10000 to 1:100000

For PVDF membrane the use of higher dilutions may be necessary.

The extract 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 - exposed 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 rabbit immunoglobulins, we have found in our laboratories that a dilution of 1:9000 is suitable for the detection of 1 μ g 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
Hybond ECL membrane	RPN 2020D
Hybond -P PVDF membrane	RPN 2020F
Hybond ECL	RPN 2103/2104/1681/1674
ECL protein molecular weight markers	RPN 2107

References

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- 1) WHITEHEAD, T.P. *et al.*, *Clin. Chem.*, **25**, pp.1531-1546, 1979.
- 2) AKHAVEN-TAFTI, H. *et al.*, *Clin. Chem.*, **41**, pp. 1368-1369, 1995.
- 3) AKHAVEN-TAFTI, H. *et al.*, *Biolum. and Chemilum. Fundamentals and Applied Aspects*, pp.199-202, Chichester, 1994.

Lumigen PS-3 detection reagent is manufactured for Amersham Biosciences Limited by Lumigen Inc. This component is covered by US Patent Nos. 5,491,072 and 5,593,845 and is sold under licence from Lumigen Inc

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<http://www.amershambiosciences.com>

Amersham Biosciences UK Limited Amersham Place
Little Chalfont Buckinghamshire England HP7 9NA

Amersham Biosciences AB SE-751 84 Uppsala Sweden

Amersham Biosciences Inc 800 Centennial Avenue PO Box 1327 Piscataway NJ08855 USA

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