



Product specification

ECL Plus Western blotting reagent pack RPN 2124

Before using this product, please read the instructions below for safe handling, storage and disposal.

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 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.

Description

This kit contains reagents for membrane blocking and secondary detection of membrane-bound mouse and rabbit primary antibodies. Detection reagents are not included, but the system is optimized for use with ECL PlusTM (1,2)

Contents of kit

Anti mouse IgG, peroxidase linked whole antibody (from sheep)	100µl
Anti rabbit IgG, peroxidase linked whole antibody (from donkey)	100µl
ECL TM Membrane blocking agent	5g

Detection of membrane bound primary antibodies

Prepare samples according to experimental requirements, following usual gel electrophoresis and western blotting techniques. We recommend use of HybondTM ECL membrane (RPN2020D) or Hybond P PVDF membrane (RPN 2020P) for maximum sensitivity. To avoid membrane contamination or damage, always use forceps and wear gloves when handling membranes.

Protocol

1) Block the membrane

Non-specific binding sites are blocked by immersing the membrane in a 5% (w/v) solution of the blocking agent in PBS or TBS Tween™ (0.1% is usually sufficient) for 1 hour at room temperature on an orbital shaker.

2) Washing

PBS or TBS Tween (0.1%) is recommended as the wash buffer. Briefly rinse the membrane twice, then wash 3 times, once for 15 minutes and twice for 5 minutes at room temperature with fresh changes of wash buffer on an orbital shaker.

3) Dilution of the primary antibody

During the washing step, dilute the primary antibody. Incubate the washed membrane in the diluted antibody. 1 hour at room temperature is often sufficient, but this should be optimized for each antibody.

4) Washing

Wash the membrane as detailed above.

5) Incubation with the species specific peroxidase linked second antibody

These dilution factors are meant as a guide only, and are indicated as suitable for the system in which they were used. Users should optimize their own system to determine the dilution factors required. It is likely that when using ECL Plus, dilution factors may need to be greater than previously used with other detection systems.

- **For detection with ECL Plus when using Hybond ECL membrane**

We have found in our laboratories that dilutions of 1:25000 of the peroxidase linked anti mouse antibody is suitable to detect 3ng of actin protein with an exposure time of 5 minutes. We have also found that a dilution factor of 1:100000 of the peroxidase linked anti rabbit antibody is suitable for the detection of 6ng of beta galactosidase with an exposure time of 5 minutes.

- **For detection with ECL Plus when using Hybond PVDF membrane**

We have found in our laboratories that dilutions of 1:50000 of the peroxidase linked anti mouse antibody is suitable to detect 3ng of actin protein with an exposure time of 5 minutes. We have also found that a dilution factor of 1:200000 of the peroxidase linked anti rabbit antibody is suitable for the detection of 6ng of beta galactosidase with an exposure time of 5 minutes.

6) Washing

Wash the membrane as detailed above.

7) Detection

Follow the detection procedures as outlined in the protocol booklet provided with the detection reagents.

Notes

- 1) It is essential to optimize both primary and secondary antibodies for results with high signal and low background due to the extreme sensitivity of the detection system when using ECL and ECL Plus.
- 2) As a general rule, as large a volume of washing buffer as possible should be used each time. It may be necessary to adjust the blocking conditions for certain applications.
- 3) Do not use azide as a preservative for buffers to be used in immunodetection as it is an inhibitor of horseradish peroxidase.

Additional information

Reagent specifications

- **Peroxidase labelled anti species antibodies**

Horseradish peroxidase conjugated antibody is 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.

Store at 2-8°C, do not freeze. Under these conditions, the reagent is stable for 6 months.

- **ECL blocking reagent**

This reagent has been selected to give effective blocking of membranes in virtually all blotting contexts, if used as directed. Caution: Moisture will affect the solubility properties of the powder. Store the container at room temperature, ensuring that the lid is tightly closed.

References

- (1) AKHAVEN-TAFTI, H. *et al.*, *Clin. Chem.*, **41**, pp.1368-1369, 1995.
- (2) AKHAVEN-TAFTI, H. *et al.*, *Biolum. and Chemilum. Fundamentals and Applied Aspects*, pp.199-202, Chichester, 1994.

Related products

ECL Plus Western blotting detection reagents	
- sufficient for 3000cm ² membrane	RPN 2133
- sufficient for 1000cm ² membrane	RPN 2132
ECL Western blotting detection reagents	
- sufficient for 6000cm ² membrane	RPN 2134
- sufficient for 4000cm ² membrane	RPN 2106
- sufficient for 2000cm ² membrane	RPN 2209
- sufficient for 1000cm ² membrane	RPN 2109
ECL Western blotting analysis system	RPN 2108
For the detection of either mouse or rabbit membrane bound primary antibodies with ECL detection reagents. Sufficient for 1000cm ² membrane	
ECL streptavidin-HRP and blocking reagent	RPN 2195
Mouse IgG, horseradish peroxidase linked whole antibody (from sheep)	NA 931
Rabbit IgG, horseradish peroxidase linked whole antibody (from donkey)	NA 934
Rat IgG, horseradish peroxidase linked whole antibody (from sheep)	NA 932
Human IgG, horseradish peroxidase linked whole antibody (from sheep)	NA 933
Mouse IgG, horseradish peroxidase linked F(Ab') ₂ fragment (from sheep)	NA 9310
Rabbit IgG, horseradish peroxidase linked F(Ab') ₂ fragment (from donkey)	NA 9340
Rat IgG, horseradish peroxidase linked F(Ab') ₂ fragment (from sheep)	NA 9320
Human IgG, horseradish peroxidase linked F(Ab') ₂ fragment (from sheep)	NA 9330
Streptavidin horseradish peroxidase conjugate	RPN 1231
Streptavidin biotinylated horseradish peroxidase complex	RPN 1051
ECL protein molecular weight markers	RPN 2107
Rainbow™ coloured protein molecular weight markers (molecular weight range 2350 – 46000)	RPN 755
Rainbow coloured protein molecular weight markers (molecular weight range 14300 - 200000)	RPN 756
Full Range Rainbow recombinant protein molecular weight markers (molecular weight range 10000 – 250000)	RPN 800
Hybond ECL nitrocellulose membrane	RPN 2020D
Hybond PVDF membrane	RPN 2020P
Hyperfilm™-ECL	RPN 2103

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