Product specification

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

ECL blocking agent
RPN 2125

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 case of contact with skin or eyes, wash immediately with water.

Quantity 40g

For blocking nitrocellulose and PVDF membranes to be used with ECL and ECL Plus™ Western blotting detection reagents.

Storage and stability

On receipt store the container at 2-8°C. After first use store at room temperature, ensuring that the lid is tightly closed. It is stable for at least six months when stored under the recommended conditions.
Caution: Moisture will affect the solubility properties of the powder. This reagent has been selected to give effective blocking of membranes in virtually all blotting contexts, if used as directed.

Quality control

This product is tested according to our quality control systems using an ECL or ECL Plus Western blotting application.

Amersham Biosciences

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. These conditions have been found in our laboratories to be sufficient for most purposes, but may need some optimization.

2) Washing
PBS or TBS Tween (0.1%) (PBS-T/TBS-T) 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) Detection
Detect the binding of the primary antibody according to usual protocols.

Notes
1) It is essential to optimize both primary and secondary antibodies for results with high signal and low background when using ECL and ECL Plus, due to the extreme sensitivity of the detection reagents.
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 if HRP linked antibodies are to be used, as it is an inhibitor of horseradish peroxidase.

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 lined F(Ab′)$_2$ fragment (from sheep)	NA 9310
Rabbit IgG, horseradish peroxidase lined F(Ab′)$_2$ fragment (from donkey)	NA 9340
Rat IgG, horseradish peroxidase lined F(Ab′)$_2$ fragment (from sheep)	NA 9320
Human IgG, horseradish peroxidase lined 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

For details of sizes, availability and ordering information, please contact your local sales office.

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