

Amersham **ECL** blocking agent

Product Specification Sheet

Introduction

Product code

RPN2125

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

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

Storage

On receipt store the container at $2-8^{\circ}$ C. After first use store at ambient temperature, ensuring that the lid is tightly closed. It is stable for at least six months when stored under the recommended conditions.

Note: 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.

Expiry

See outer packaging.

Components

Quantity: 40 g.

For blocking nitrocellulose and PVDF membranes to be used with ECL $^{\text{TM}}$ and ECL Plus Western blotting detection reagents.

Quality control

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

Protocol

Step Action

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.

Step Action

- 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. I 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.

Note: 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.

Note: 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.

Note: Do not use azide as a preservative for buffers to be used in immunodetection as it is an inhibitor of Horseradish Peroxidase.

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Related products

| RPN2133 | |
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| RPN2132 | |
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| RPN2134 | |
| RPN2106 | |
| RPN2209 | |
| RPN2109 | |
| RPN2108 | |
| ne bound | |
| primary antibodies with ECL detection reagents. Sufficient for 1000 cm ² membrane | |
| DDNO105 | |
| RPN2195 | |
| NA931 | |
| NA934 | |
| NA932 | |
| NA933 | |
| NA955 | |
| NA9310 | |
| NA9340 | |
| NA9320 | |
| NA9330 | |
| RPN1231 | |
| RPN1051 | |
| RPN2107 | |
| RPN755 | |
| 1111755 | |
| RPN756E | |
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| Full Range Rainbow recombinant protein molecular weight markers (molecular weight range 12 000 – 225 000) | RPN800E |
|---|-------------------|
| Hybond™ ECL nitrocellulose membrane | RPN2020D |
| Hyperfilm™-ECL | RPN2103 |
| For details of sizes, availability and ordering inf contact your local sales office. | formation, please |

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