

# Product specification

## ECL *in vitro* translation - streptavidin-HRP and blocking reagent

code RPN 2195

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

**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 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 detection of membrane-bound biotinylated products with streptavidin-HRP conjugate.

## Contents of kit

Streptavidin-horseradish peroxidase conjugate	0.5ml
Membrane blocking agent	40g

## Detection of biotinylated translation products.

Following translation in the presence of biotin-lys-tRNA to give biotinylated proteins, the products are separated by SDS-PAGE and blotted on to nitrocellulose membrane. The immobilized biotinylated proteins are detected by incubation with streptavidin-HRP and visualized with ECL<sup>TM</sup> detection reagents.

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 5% blocking agent/PBS-T (see Appendix II) for 1 hour at room temperature on an orbital shaker.

## 2) Washing

PBS-0.1% Tween™ 20 (PBS-T) should be used for the washing buffer (see Appendix II). Briefly rinse the membrane twice with PBS-T then wash 3 times in PBS-T, once for 15 minutes and twice for 5 minutes at room temperature with fresh changes of wash buffer on an orbital shaker.

## 3) Streptavidin-HRP

Dilute the streptavidin-HRP conjugate 1/1000 in PBS-T and incubate the membrane for 1 hour at room temperature on an orbital shaker.

## 4) Washing

Briefly rinse the membrane twice with PBS-T then wash 3 times in PBS-T, once for 15 minutes and twice for 5 minutes at room temperature with fresh changes of washing buffer on an orbital shaker.

## 5) Signal generation and detection.

Follow the detection procedures as outlined in the "ECL Western blotting" protocol booklet provided with ECL detection reagents.

## Notes

2.1) As a general rule, as large a volume of washing buffer as possible should be used each time.

3.1) It is strongly advised that blocking agent is not included in the streptavidin-HRP incubation. The binding of streptavidin-HRP to biotin is inhibited by the presence of milk<sup>(1)</sup> resulting in a reduced signal when detected by enhanced chemiluminescence.

## Appendix I.

### Reagent specifications

- Streptavidin-HRP

## Preparation

The conjugation of horseradish peroxidase and streptavidin is achieved using the periodate coupling method described by Nakane and co-workers<sup>(2)</sup>. This product is sold on the basis of titre in a standard assay. As a result the final protein concentration on the conjugate will vary only minimally between batches.

## Quality control

For every batch of peroxidase labelled streptavidin that is produced an ELISA is used to determine the titre of the reagent. The substrate used for the peroxidase is 2,2'-azino-di-[3-ethylbenzthiazoline sulphonate] (ABTS<sup>TM</sup>).

## Formulation

The conjugate is supplied in 0.5ml of phosphate buffered saline (0.1M sodium phosphate, 0.1M sodium chloride pH 7.4) containing bovine serum albumin (1% w/v) and an antimicrobial agent.

## Storage and stability

Store at 2-8°C. Under these conditions the product is stable for at least 6 months from the date of despatch as judged by its ability to detect biotinylated protein immobilized on plastic microtitre plates.

### • Membrane blocking agent

This reagent has been selected to give effective blocking of nitrocellulose 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. Under these conditions the product is stable for at least 6 months from the date of despatch.

## Appendix II.

### Buffer preparation and storage.

#### 1) Phosphate-buffered saline (PBS) pH 7.5

11.5g di-sodium hydrogen orthophosphate anhydrous	(80mM)
2.96g sodium dihydrogen orthophosphate	(20mM)
5.84g sodium chloride	(100mM)

Dissolve in 800ml of distilled water - dilute to final volume of 1000ml. Check pH.

## 2) PBS/0.1% Tween-20 (PBS-T)

Add Tween-20 to PBS to give a concentration of 0.1% Tween-20, e.g. 1ml of Tween-20 added to 999ml of PBS.

PBS-T should be stable for at least 3 months stored at room temperature, although storage in a refrigerator (2-8°C) may be necessary to avoid microbial growth. Sodium azide should not be used as a bactericide.

## 3) Membrane blocking solution (5% blocking agent/PBS-T)

Blocking solution should be prepared fresh. Dissolve 2.5g of blocking agent in 35ml of PBS-T. Make the volume up to 50ml with PBS-T.

## References

(1) HOFFMAN, W.L. and JUMP, A.A., *Anal. Biochem.*, **191**, pp.318-320, 1989

(2) WILSON, M.B. and NAKANE, P.K., pp.215-244 in *Immunofluorescence and Related Staining Techniques*, edited by KNAPP, W. et al, Elsevier North Holland, 1978

## Associated products

ECL Western blotting detection reagents

- sufficient for 4000cm<sup>2</sup> membrane

RPN 2106

- sufficient for 2000cm<sup>2</sup> membrane

RPN 2209

- sufficient for 1000cm<sup>2</sup> membrane

RPN 2109

ECL Western blotting analysis system

RPN 2108

For the detection of either mouse or rabbit membrane bound primary antibodies.

Sufficient for 1000cm<sup>2</sup> membrane

ECL protein molecular weight markers

RPN 2107

Rainbow™ coloured protein molecular weight markers

RPN 755

(molecular weight range 2350 - 46000)

Rainbow coloured protein molecular weight markers

RPN 756

(molecular weight range 14300 - 200000)

Hybond™ECL nitrocellulose membrane

RPN 2020D

Hyperfilm™ECL

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

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