

# Product specification

## ECL protein molecular weight markers RPN 2107

### 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.

Warning: Potentially infectious material.

Human blood products provided as components of this pack have been obtained from donors who were tested individually and who were found to be negative for the presence of Human Immunodeficiency Virus antibody (HIV-Ab)\* as well as for Hepatitis B surface Antigen (HBsAg) using approved methods (Enzymeimmunoassay).

As no test method can offer complete assurance that Hepatitis B virus, Human Immunodeficiency Virus antibody (HIV-Ab)\* or other infectious agents are absent, all human blood products should be considered potentially infectious. Handling, use, storage and disposal should be in accordance with the procedures defined by an appropriate National biohazard safety guideline or regulation, where it exists (for example USA Centre for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories", 2nd Edition, 1988).

\*HIV is the abbreviation used for HTLV-III and LAV.

Warning: Contains sodium azide. See safety data sheet supplied.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is 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. 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 (see safety data sheet for specific advice).

### Description

ECL<sup>TM</sup> protein molecular weight markers from Amersham Biosciences are a mixture of six different proteins labelled with biotin for use in Western blotting following electrophoresis on a polyacrylamide gel prepared by the method of Laemmli<sup>(1)</sup>. Incubation of the blot with streptavidin horseradish peroxidase followed by detection with the ECL Western blotting detection system will result in a ladder of bands, on film, of approximately equal intensity.

The molecular weights of these standards are not significantly altered by biotinylation.

To utilize the ECL biotinylated markers, it is essential to incubate the blot with streptavidin-HRP conjugate. Order product code RPN 2280 to receive the markers with streptavidin-HRP and blocking reagent.

## Formulation

Supplied in phosphate buffered saline containing 50% glycerol, 0.2% sodium azide. See safety data sheet.

## Concentration

1mg/ml total protein.

## Specification

The product contains the following proteins:

Phosphorylase b (rabbit muscle)		MW 97000
Bovine serum albumin		MW 66000
Ovalbumin (hen egg white)		MW 45000
Carbonic anhydrase (human erythrocytes)		MW 31000
Trypsin inhibitor (soybean)		MW 20100
Lysozyme (hen egg white)		MW 14400

**Figure 1. Profile of ECL protein molecular weight markers**

1 $\mu$ l sample ECL protein molecular weight markers diluted with 9 $\mu$ l of loading buffer and run on a 12% polyacrylamide gel for 1 hour at 150 volts, followed by electroblotting on to Hybond ECL overnight at 30 volts. Processing of the blot was as outlined in the ECL Western blotting protocol, using Streptavidin-HRP (RPN 1231, 1:1500) dilution) and ECL Western blotting detection reagents. The light emission was captured using Hyperfilm ECL for a 15 second exposure.

Due to the sensitivity of this system, further faint bands may be observed at approximate MW of 55000, 43000 and 16700.

## Storage

Store at -15°C to -30°C.

## Pack size

25 $\mu$ l.

## Use of ECL protein molecular weight markers

The following protocol is recommended:

1. Remove 1 $\mu$ l of markers and add to 9 $\mu$ l of gel loading buffer (containing 5% 2- $\beta$ -mercaptoethanol).
2. Heat to 100°C for 4 minutes. Samples may be loaded on to the gel immediately, or stored temporarily on ice.
3. Load 10 $\mu$ l per well.
4. Following electrophoresis and transfer to nitrocellulose membranes, blots are processed by standard immunodetection protocols as outlined in the ECL Western blotting detection protocol booklet. If the protocol used is not a biotin-streptavidin system, then streptavidin-HRP (RPN 1231) is added (1:1500) in the final antibody incubation. It is strongly advised that milk should not be included in the streptavidin-HRP incubation. The binding of streptavidin to biotin is inhibited by the presence of milk<sup>(2)</sup>, resulting in a much reduced signal when detected by enhanced chemiluminescence. If cross reactivity is observed between the streptavidin-HRP and protein samples on the blot, it is suggested that the lane containing the markers is removed and incubated in streptavidin-HRP separately. The strip can then be re-aligned with the rest of the blot for ECL detection.
5. Follow the recommended wash and detection procedures of the ECL Western blotting detection products protocol (RPN 2106, RPN 2108, RPN 2109).
6. The volume of markers required to give optimum results will depend on the electroblotting and immunodetection conditions used and the length of exposure to film required.

As a guideline a 10 $\mu$ l loading will produce clearly visible bands on a 15 second exposure using overnight electroblotting in Towbin buffer<sup>(3)</sup> and standard ECL Western blotting immunodetection protocols.

## Related products

Streptavidin-horseradish peroxidase conjugate (recommended dilution 1:1500)	RPN 1231
Streptavidin-biotinylated peroxidase complex (recommended dilution 1:1500)	RPN 1051
HRP-conjugated second antibodies	NA931 – NA934, NXA931 (GPR)
HRP-conjugated F(ab') <sub>2</sub> fragments	NA9310 – NA9340
ECL Western blotting detection reagents	
• Sufficient reagents for 4000cm <sup>2</sup> membrane	RPN 2106
• Sufficient reagents for 1000cm <sup>2</sup> membrane	RPN 2109
• Sufficient for 6000cm <sup>2</sup> membrane	RPN 2134
• Sufficient for 2000cm <sup>2</sup> membrane	RPN 2209
ECL Plus™ Western blotting detection system	RPN 2132
• Sufficient for 1000cm <sup>2</sup> membrane	
ECL Plus Western blotting detection system	RPN 2133
• Sufficient for 3000cm <sup>2</sup> membrane	
ECL Western blotting analysis system	RPN 2108
• Sufficient for 1000cm <sup>2</sup> membrane (includes HRP-conjugated second antibodies)	
ECL Plus Western blotting reagent pack	RPN 2124
ECL blocking reagent	RPN 2125

Hybond™ ECL nitrocellulose membrane

Hyperfilm™ ECL paperbased alternative to Hyperfilm ECL

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

## References

- 1) LAEMMLI, U.K., *Nature*, **227**, p.681, 1970.
- 2) HOFFMAN, W.L. and JUMP, A.A., *Anal. Biochem.*, **181**, p.318, 1989.
- 3) TOWBIN, H. *et al.*, *Proc. Natl. Acad. Sci.*, **76**, p.4350, 1979.

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