Amersham **ECL DualVue** Western Blotting Markers

Product Specification Sheet

Introduction

Product code

RPN810

About

Molecular weight range 15 000-150 000

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.

This product is used in conjunction with gel electrophoresis. Please follow the manufacturers instructions relating to the handling and use of the equipment and materials.

Storage

Store S-protein-HRP conjugate at -15°C to -30°C. Store DualVue Western blotting markers at -15°C to -30°C. Ensure conjugate and markers are returned to the freezer immediately after each use.

Expiry

Stable for three months when stored under recommended conditions.

Components

- 125 µL ECL DualVue™ Western blotting markers
- 50 µL S-protein-HRP conjugate

The kit contains sufficient components for marker detection on 25 blots (10 cm X 10 cm; 10 mL incubation volumes using Hybond™ ECL™ nitrocellulose membrane in conjunction with ECL detection reagents).

Form

ECL DualVue Western blotting markers are supplied in 35% glycerol and sample buffer containing mercaptoethanesulphonic acid (MESNA) as a reducing agent (2).

Description

ECL DualVue Western blotting markers consist of a combination of two types of protein marker:

- Pre-stained indicator proteins that confirm blot transfer and blot orientation
- Recombinant tagged proteins that ensure accurate molecular mass determination of the target protein(s) following chemiluminescent detection on film or by CCD imaging

There are three pre-stained indicator proteins with approximate molecular masses of 15 kDa (red), 16 kDa (blue) and 100 kDa (red). These three coloured bands are clearly visible following transfer to membrane providing a reliable indicator of protein transfer (see A in *Fig. 1, on page 2*). Also, blot orientation is apparent at all times. These proteins are not tagged and therefore not subsequently detected by chemiluminescent substrates.

These indicator proteins are supplemented with a set of seven recombinant protein markers with precise molecular masses that each contain a tagged peptide sequence. The marker set is easily and specifically detected by binding S-protein-HRP conjugate and developing with chemiluminescent substrates. Since no chemical modification is required to label the marker proteins, their migration accurately represents their sizes when separated on a polyacrylamide gel as described by Laemmli (1).

The molecular masses of the recombinant tagged proteins are 15, 25, 35, 50, 75, 100 and 150 kDa (see B in *Fig. 1, on page 2*). The migration and band sharpness of the recombinant tagged proteins are unaffected by the presence of the pre-stained indicator proteins.

ECL DualVue Western blotting markers cannot be used in conjunction with full range Rainbow™ markers (Product code RPN800) since the proteins used for this product also contain the tagged region. However, the product is compatible with high or low range Rainbow markers (Product codes RPN756 and RPN755 respectively).

Protocol

The S-protein-HRP conjugate can be added during either the primary or secondary antibody incubation. The protocol below includes addition of the conjugate during the secondary antibody incubation.

Step	Action
1	Remove the ECL DualVue Western blotting markers from storage at -15°C to -30°C and allow equilibration to room temperature. A precipitate of SDS may form on storage at -15°C to -30°C. If necessary briefly warm the solution at 37°C to dissolve the precipitate.
2	Mix well and add 5 µL of marker to an equal volume of loading buffer containing 10% Beta-mercaptoethanol (or a loading buffer containing an equivalent reducing agent). Perform electrophoresis according to standard techniques.

Step	Action
3	Transfer the proteins electrophoretically to Hybond ECL or Hybond PVDF for optimum results. Any standard blotting device can be used according to the manufacturer's instructions. The transferred pre-stained marker proteins should be visible on the membrane after transfer.
4	Process the blot according to your standard protocol for blocking and primary antibody incubation steps.
5	Incubate the membrane with your secondary antibody at the required dilution. To this solution add the S-protein-HRP conjugate at the appropriate dilution for the system being used (as indicated in *Table 1, on page 2*). A minimum incubation of 30 minutes at room temperature is recommended for maximum signal generation.
6	Wash the membrane according to standard protocols.
7	Visualize the proteins using chemiluminescent substrates according to manufacturer's instructions. Initial film exposure times of 1 and 2.5 minutes are recommended.

Table 1. Recommended S-protein-HRP conjugate dilutions when using Cytiva's chemiluminescent detection reagents with Hybond membranes and detection on film.

Chemiluminescent Detection Reagent	Hybond ECL Nitrocellulose Membrane	Hybond-P PVDF Membrane
ECL	1/5 000	1/10 000
ECL Plus	1/10 000	1/20 000
ECL *Advance*™	1/100 000	1/200 000

Note: *For CCD camera detection the S-protein-HRP conjugate concentration needs to be determined empirically - as a guide a 10- fold increase in concentration is recommended.*

Typical results

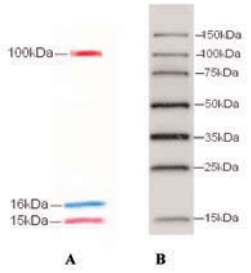

Fig 1. ECL DualVue Western Blotting markers after electrophoresis on a 4-20% SDS-PAGE slab gel and transfer onto Hybond ECL nitrocellulose membrane showing pre-stained indicator proteins (A) and tagged proteins after detection with ECL Western blotting detection reagents (B). Electrophoresis performed for 45 minutes at 200 volts, 1 minute film exposure.

Quality control

Each batch of ECL DualVue Western blotting markers is assessed for colour intensity of the pre-stained protein markers on transfer to membrane. The tagged proteins are assessed for band integrity and intensity by detection with ECL on Hybond ECL nitrocellulose membrane.

The 15 000 and 150 000 Da bands may give weaker signals than the other species depending on the percentage gel, membrane support or transfer conditions used.

Under certain conditions a very weak pre-stained protein band at 35 000 Da may be observed on the membrane following transfer.

Related products

Cytiva offers a comprehensive range of Western blotting reagents and hardware all with proven compatibility to ensure reproducible high quality results. For a complete listing of products available see the current Cytiva catalogue or visit our web site at *cytiva.com*.

ECL Western Blotting Detection Reagents for 4000 cm^2 membrane	RPN2106
ECL Plus Western Blotting Detection Reagents for 1000 cm^2 membrane	RPN2132
ECL *Advance* Western Blotting Detection Kit Other pack sizes and detection reagents also available	RPN2135
Low-range Rainbow MW markers	RPN755
High-range Rainbow MW markers	RPN756
Full-range Rainbow MW markers (recombinant)	RPN800
ECL Western Blotting MW markers, biotinylated	RPN2107
Hybond-P membrane (PVDF, pore size 0.45 µm)	RPN2020F
Hybond-ECL membrane (nitrocellulose, pore size 0.45 µm)	RPN303D
Other membrane sizes also available	
Hyperfilm™ ECL 18 x 24 cm, pack of 25 films Other film sizes are also available	RPN2103K
Streptavidin-biotinylated horseradish peroxidase complex	RPN1051
Streptavidin horseradish peroxidase conjugate	RPN1231
Mouse IgG, HRP linked whole antibody (from sheep), 1 mL	NA931
Rabbit IgG, HRP linked whole antibody (from sheep), 1 mL.	NA934
Other conjugates are also available	

References

1. Laemmli, U.K. *Nature*, 227, 681 (1970).
2. Singh, R. *Biotechniques*, 17, 263 (1994).

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