

**Renaissance Biotinylated Protein Molecular Weight Markers
NEL-310**

CONCENTRATION: 1 mg/mL total protein
PACKAGE SIZE 50 μ L
STORAGE: Store at -20°C
PACKAGING: Supplied in 10mM TRIS-HCl, pH 8.8/50% glycerol, 0.009% sodium azide.

Shipped in dry ice.

SPECIFICATION:

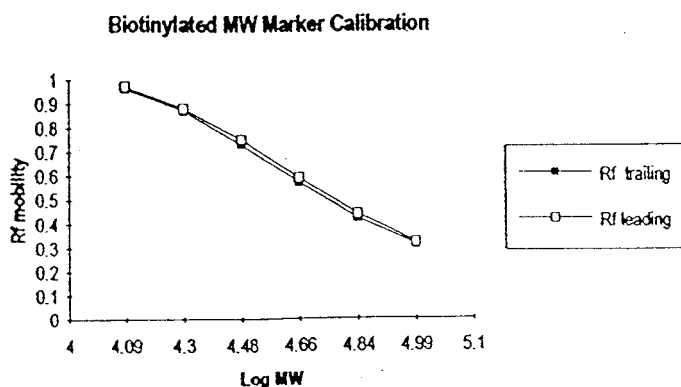
The product contains the following proteins:

| | |
|--|-----------|
| Phosphorylase b (rabbit muscle) | MW97,400. |
| Bovine serum albumin | MW69,000 |
| Ovalbumin (chicken eggwhite) | MW46,000 |
| Carbonic anhydrase (bovine erythrocytes) | MW30,000 |
| Trypsin Inhibitor (soybean) | MW20,100 |
| Cytochrome C (horse heart) | MW12,300 |

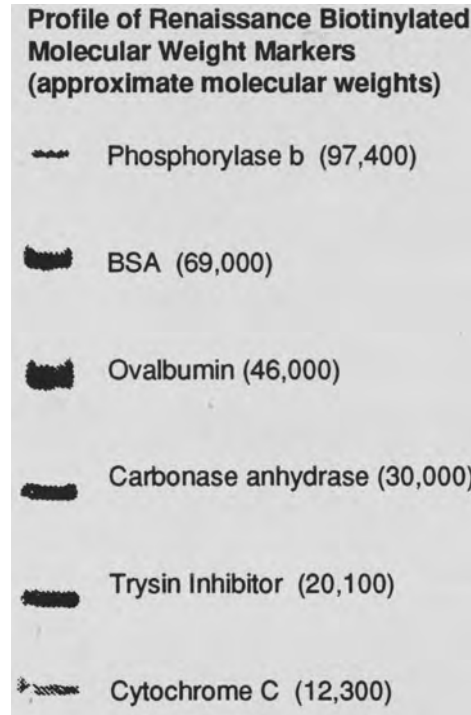
All the components of this mixture are of animal origin and do not require BSL II containment for handling.

One μ L of the protein mix is diluted in 20 μ L loading buffer (a 200 ng load is recommended on a minigel), electrophoresed by the Laemmli method (1), electroblotted onto Dupont Polyscreen™ PVDF membrane in Towbin buffer (2) and detected with the NEN® Renaissance Western Blot chemiluminescence reagent. Visible bands are produced after a 10 minute exposure to NEN Kodak X-Omat Blue film.

The following calibration curve was determined with a 10 μ L loading on a 4%-20% SDS gel, electroblotted in Towbin buffer and detected with the standard NEN Renaissance Western Blot chemiluminescence protocol.



SDS PAGE profile: One microliter of Biotinylated Molecular Weight Markers was diluted in 20 μ L of loading buffer, electrophoresed by the Laemmli method (1), electroblotted onto PolyScreen PVDF Transfer Membrane in Towbin Buffer (2) and detected with WesternLightning Chemiluminescence reagent. The membrane was then exposed to X-ray film for ten minutes.



RELATED PRODUCTS:

| | |
|--|------------------|
| Streptavidin-HRP Conjugate | NEL750 |
| Streptavidin-AP Conjugate | NEL751 |
| WesternLightning PLUS | NEL103 - NEL105 |
| WesternLightning CDP-Star | NEL602 |
| PolyScreen [®] PVDF Transfer Membrane | NEF1000, NEF1002 |

REFERENCES:

1. Laemmli, U.K., Nature, 227, p.681, 1970
2. Towbin, H., et al., Proc. Nat. Acad. Sc., 76, p.4350, 1979.

SIMPLIFIED WESTERN BLOTTING PROTOCOL

- A. It is recommended that the transfer buffer (Towbin transfer buffer, pH 8.3) be made up ahead of time and pre-cooled to 4°C. In this way, it will have a chance to degas before use. Bubbles in the transfer buffer will increase the chance of trapping air between the membrane and the gel. Air bubbles create points of high resistance, resulting in "bald spots" (i.e., areas of low efficiency transfer and band distortions).

Towbin Transfer buffer (1)

| | |
|--------------------------------------|-----------------|
| 25 mM TRIS | 3.03g |
| 192 mM Glycine | 14.41g |
| Add H ₂ O to make 1 liter | |
| 20% MeOH | Add 250 mL MeOH |

- B. Cut the PolyScreen™ PVDF membrane (cat #NEF-1000 & NEF-1002) slightly larger than the gel. When using PolyScreen™, prewet with methanol or 95% ethanol, then rinse with water. For nitrocellulose, just rinse with water. Be sure to wear gloves at all times when handling the membranes. Mark one side of the membrane for future reference.
- C. Equilibrate both the membrane and the gel in transfer buffer for 15-20 minutes.
- D. Wet two Scotch-Brite® pads and two pieces of filter paper (Whatman® 3MM cut to the size of the gel) in transfer buffer.
- E. Prepare the "sandwich" as follows:
- Put one piece of wet filter paper on a Scotch-Brite® pad.
 - Place the equilibrated gel on top of the filter paper.
 - Place the membrane on top of the gel.
 - Place the second piece of wet filter paper over the membrane.
 - Be sure to remove any air bubbles trapped between the gel, membrane and filter paper layers. This is easily done by rolling a clean piper over the sandwich.
 - Complete the sandwich with the second Scotch-Brite® pad.
- F. Insert the sandwich into the transfer apparatus with the membrane positioned between the gel and the appropriate electrode. Most polypeptides are eluted from SDS-polyacrylamide gels as anions and therefore the membrane should usually be placed between the gel and the anode.
- G. Fill the transfer apparatus with buffer. Pour the transfer buffer slowly to prevent bubble formation. Cool to 4°C and transfer at a constant current or voltage.
- H. When the transfer is complete, remove the membranes and allow them to air dry at room temperature. Since dehydrated proteins bind more strongly to the membrane, this helps to prevent loss of target during subsequent washes.
For more instructions, please refer to the Manual provided with the NEN RENAISSANCE™ WESTERN BLOT CHEMILUMINESCENCE REAGENT (cat# NEL-100, NEL-101, NEL-102).

(1) PNAS 76: 4340-4354, 1979

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Whatman® is a trademark of Whatman Paper Ltd.