

ScanLater™ Western Blot Protein Ladder Reagent

The ScanLater Western Blot Protein Ladder Reagent contains seven biotinylated proteins in the molecular weight range of 10 kD to 140 kD to be detected using Eu-labeled streptavidin. In addition, three blue-stained protein bands are visibly detected at 18 kD, 31 kD, and 70 kD to monitor migration during gel electrophoresis and to assess the efficiency of protein transfer.

The ScanLater™ Western Blot Protein Ladder Reagent is designed to be used with the ScanLater™ Western Blot Detection System.

Table 1-1: Ordering Information

Reagent	Part Number
ScanLater™ Western Blot Protein Ladder Reagent (130 µL)	R8220

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ScanLater Western Blot Protein Ladder Reagent

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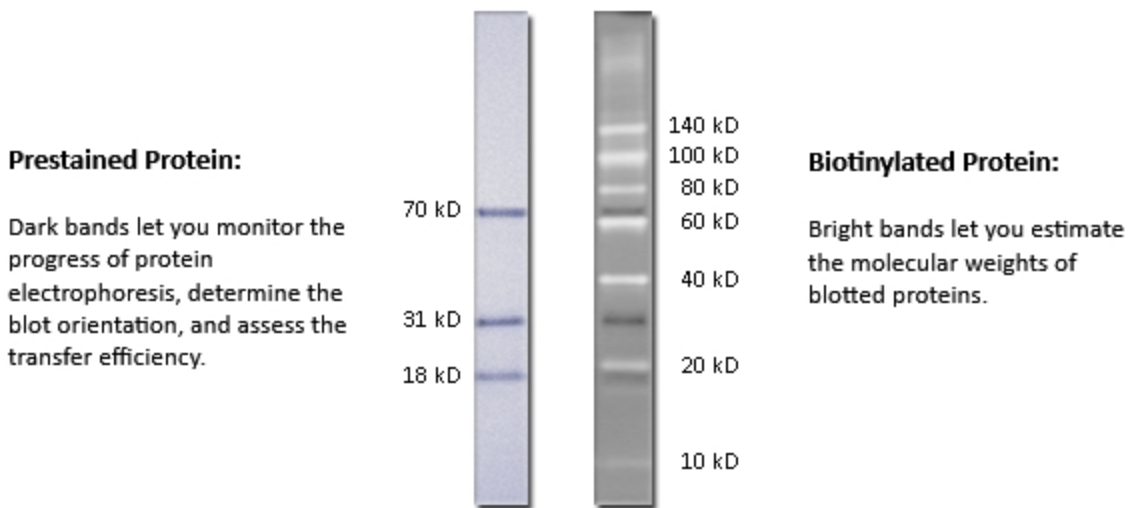
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Chapter 1: About the ScanLater Western Blot Protein Ladder Reagent

The ScanLater™ Western Blot Protein Ladder Reagent is designed to be used with the ScanLater™ Western Blot Detection System.

Assay Principle

The ScanLater Western Blot Protein Ladder Reagent contains seven biotinylated proteins in the molecular weight range of 10 kD to 140 kD to be detected using Eu-labeled streptavidin. The Western Blot is probed with a secondary antibody combination of Eu-streptavidin and the Eu-labeled secondary antibody required by your primary antibody. These components can be ordered separately from Molecular Devices.



In the blot, 10 kD, 20 kD, 40 kD, 60 kD, 80 kD, 100 kD, and 140 kD biotinylated bands are detected with the Eu-Labeled streptavidin. In addition, three blue-stained protein bands are visibly detected at 18 kD, 31 kD, and 70 kD to monitor migration during gel electrophoresis and to assess the efficiency of protein transfer.

Chapter 2: Materials and Equipment

Package Contents

Table 2-1: Contents of the ScanLater Western Blot Protein Ladder Reagent Package

Reagent	Part Number
ScanLater™ Western Blot Protein Ladder Reagent (130 µL)	R8220

The reagent contains loading buffer with the following components:

- 30% glycerol (weight/volume)
- 2% SDS
- 2 M urea
- 62.5 mM Tris, pH 6.8
- 6 mM EDTA
- 0.02% NaN₃
- 0.01% Bromophenol blue

The Western Blot is probed with a combination of Eu-labeled streptavidin and the Eu-labeled antibody required for your secondary antibody. These components can be ordered separately from Molecular Devices.

Table 2-2: Components that can be Ordered Separately

Item	Volume	Part Number
ScanLater 10X Washing Buffer	100 mL	R8206
ScanLater 5X Blocking Buffer	120 mL	R8207
ScanLater Eu-Labeled Goat Anti-Mouse IgG	60 µg	R8208
ScanLater Eu-Labeled Goat Anti-Rabbit IgG	60 µg	R8209
ScanLater Eu-Labeled Donkey Anti-Goat IgG	60 µg	R8226
ScanLater Eu-Labeled Donkey Anti-Rat IgG	60 µg	R8229
ScanLater Eu-Labeled Streptavidin	60 µg	R8212

Materials Required but not Provided

To use the ScanLater Western Blot Protein Ladder Reagent, you need the following instrumentation and supplies from Molecular Devices:

- SpectraMax Paradigm or SpectraMax i3 Multi-Mode Detection Platform
- ScanLater Western Blot (WB) Detection Cartridge
- ScanLater Membrane Holder
- Western Blot membranes

For optimal results, use EMD Millipore Immobilon FL (IPFL00010).

Storage and Handling

On receipt of the ScanLater Western Blot Protein Ladder Reagent, store the contents at -20°C . Under these conditions the reagent is stable for six months in the original packaging. For best performance, store the contents at -80°C .



WARNING! Reagents can contain chemicals that are harmful. Exercise care when handling reagents as described in the related safety data sheet (SDS or MSDS). The safety data sheet is available in the Knowledge Base on the Molecular Devices support web site:

www.moleculardevices.com/support.html

Supported Instruments

The ScanLater Western Blot Protein Ladder Reagent is designed to be used with the following instruments:

- SpectraMax® Paradigm® Multi-Mode Detection Platform
- SpectraMax® i3 Multi-Mode Detection Platform

The instrument must have the ScanLater Western Blot (WB) Detection Cartridge installed to scan the membrane.

Chapter 3: Experimental Protocol

Instructions for Using the ScanLater Western Blot Protein Ladder Reagent

The protein ladder components are provided in the sample buffer, ready to load, with no dilution, reducing, or heating required.

1. Allow the protein ladder reagent to reach room temperature, and thoroughly mix it to make sure that any precipitates are thoroughly dissolved.
Do not heat the solution above room temperature.
2. Load 4 μ L to 8 μ L per lane on a standard mini gel.
3. Immediately return the reagent to storage at -20°C or -80°C .
4. Run electrophoresis as usual.
5. Transfer the gel to the blotting membrane as required by your application.



Note: When the gel is transferred, a prominent band at 10 kD and a prominent dye front appear along with the expected blue bands at 18 kD, 31 kD, and 70 kD. As the blot is processed, the 10 kD band and the dye front disappear, leaving only the 18 kD, 31 kD, and 70 kD blue bands.

6. Block the membrane, incubate the primary antibody, and wash the membrane as required for your application.
7. Prepare a secondary-antibody mixture by combining Eu-Labeled streptavidin and the Eu-labeled secondary antibody required by your application.
The dilution of Eu-streptavidin can be from 1:2500 to 1:10000, preferably the same dilution as you use for your secondary antibody.
8. Add the mixture to the membrane and incubate for 60 minutes.
9. Wash the membrane 3 times in 1X wash buffer for 5 minutes each time.
10. Rinse the membrane with water for 15 seconds and let it dry before scanning. The membrane can be scanned wet right after the wash step, but a dry membrane has a higher signal-to-background ratio.
11. Scan the membrane in a SpectraMax Paradigm or SpectraMax i3 Instrument with a ScanLater Western Blot (WB) Detection Cartridge installed.

Download the Complete ScanLater Western Blot Assay Kit Instructions

Complete instructions for using the ScanLater Western Blot Assay Kit for preparing the membrane and acquiring data can be downloaded from the Knowledge Base on the Molecular Devices support web site:

www.moleculardevices.com/support.html

Click **Knowledge Base**, and then click **Manuals** and search for **ScanLater** or **Western Blot**.

Chapter 4: Obtaining Support

Molecular Devices is a leading worldwide manufacturer and distributor of analytical instrumentation, software and reagents. We are committed to the quality of our products and to fully supporting our customers with the highest possible level of technical service.

Our support web site, www.moleculardevices.com/support.html, has a link to the Knowledge Base with technical notes, software upgrades, safety data sheets, and other resources. If you do not find the answers you are seeking, follow the links to the Technical Support Service Request Form to send an email message to a pool of technical support representatives.

You can contact your local representative or contact Molecular Devices Technical Support by telephone at 800-635-5577 (U.S. only) or +1 408-747-1700. In Europe call +44 (0) 118 944 8000.

Please have the product name, part number, and lot number available when you call.

Tips and Recommendations

Some of the proteins in the protein ladder reagent have glutathione S-transferase (GST), V5 sequence, and maltose-binding protein (MBP) in their sequence. If you are using primary antibodies specific to GST, V5, or MBP, these antibodies can cross-react with certain bands in the ladder. For example, a primary antibody for GST labels the ladder bands at 40 kD, 80 kD, and 140 kD making these bands more prominent, since there is GST in the sequence of these proteins.

Cell extracts can contain naturally occurring biotinylated proteins. If you suspect that your samples contain biotinylated proteins, you can cut out the lanes containing the ladder and process them separately with Eu-labeled streptavidin.

Contact Us

Phone: +1-800-635-5577
Web: www.moleculardevices.com
Email: info@moldev.com

Check our website for a current listing of worldwide distributors.

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