INSTRUCTIONS

SuperSignal® Enhanced Molecular Weight Protein Ladder

84786 2264.1

Number	Description
84786	**SuperSignal Enhanced Molecular Weight Protein Ladder,** 250µL, for use with mouse monoclonal antibodies, sufficient for 50 lanes at 5µL per lane

Storage: Upon receipt store at -20°C. Product is shipped with an ice pack.

Introduction

The Thermo Scientific SuperSignal Enhanced Molecular Weight Protein Ladder is formulated specifically for antibody species that do not bind well to other antibody-based ladders, such as mouse monoclonal. This ladder is supplied as a ready-to-use mixture of eight recombinant proteins ranging from 20K to 150K (Figure 1). Each protein in the mixture contains an IgG-binding site and is proportioned to yield comparable gel electrophoresis and Western blotting band intensities. The ladder contains a pink tracking dye in the buffer for monitoring electrophoresis and transfer. The ladder can be used with chemiluminescent, fluorescent, chromogenic and other detection types.

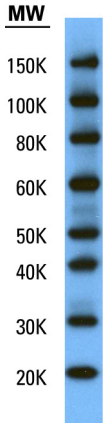

Figure 1. Chemiluminescent detection of the molecular weight protein ladder. The ladder (10µL) was applied to a 4-20% Thermo Scientific Precise Gel and transferred to PVDF membrane. The membrane was blocked with Thermo Scientific StartingBlock T20 (TBS) Blocking Buffer (Product No. 37543) and probed with anti-Cdk5 mouse monoclonal antibody (1/1,000 dilution; Thermo Scientific Lab Vision) followed by goat anti-mouse-HRP (200ng/mL; Product No. 31430). Thermo Scientific Pierce ECL Western Blotting Substrate (Product No. 32106) was used for detection.

Important Product Information

- Do not boil the marker mix.
- Store the SuperSignal Molecular Weight Protein Ladder for up to one month at room temperature, up to three months at 4ºC, or for one year at -20ºC.
- Empirically determine the volume of the ladder to use. Antibody concentrations, antibody-binding affinity, blocking buffers and substrate sensitivity will affect band detection.
- If the ladder requires diluting, use a reducing sample loading buffer (e.g., Thermo Scientific Lane Marker Reducing Sample Buffer, Product No. 39000).

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Procedure for Using the Enhanced Molecular Weight Protein Ladder

1. Load 1-10µL of the protein Ladder onto the gel. The volume to load depends on the detection method that will be used. See Table 1 for loading volume suggestions.

Table 1. Loading suggestions for various detection methods.

Detection System	Loading Volume (µL)
Colorimetric	10
Fluorescent	5-10
Chemiluminescence	1-10
ECL Substrate	5-10
SuperSignal West Pico Substrate	5-10
SuperSignal West Dura Substrate	2-5
SuperSignal West Femto Substrate	1-5

Note: The amount of marker applied to the gel may require optimization. When low volumes are required, dilute the protein ladder in reducing sample loading buffer to ensure loading consistency and adequate well coverage.

2. Stop the electrophoresis when the pink dye front has reached the bottom of the gel.
3. Proceed with the transfer and Western blot detection.

Note: Store the SuperSignal Molecular Weight Protein Ladder for up to one month at room temperature, up to three months at 4ºC, or for one year at -20ºC.

Troubleshooting

Problem	Possible Cause	Solution
No bands of the protein standard were detected	Loading volume was insufficient	Use a greater volume of the ladder
	Transfer was incomplete	Optimize transfer conditions
Bands of the protein standard were oversaturated (i.e., no visible band separation)	Antibody concentrations were too high for the amount of standard loaded onto the gel	Use less volume of the ladder
	The protein ladder was boiled	Do not boil the ladder

Related Thermo Scientific Products

Please see the catalog or website for a complete listing of protein gels and Western blotting products.

21059 **Restore™ Western Blot Stripping Buffer,** 500mL

46430 **Restore PLUS Western Blot Stripping Buffer,** 500mL

88600 **Western Blotting Filter Paper, 8cm × 10.5cm,** 100 sheets

32106 **Pierce ECL Western Blotting Substrate,** 500mL

34080 **SuperSignal West Pico Chemiluminescent Substrate,** 500mL

34075 **SuperSignal West Dura Extended Duration Substrate,** 100mL

34095 **SuperSignal West Femto Maximum Sensitivity Substrate,** 100mL

34090 **CL-XPosure™ Film (5" × 7"),** 100 sheets

34091 **CL-XPosure Film (8" × 10"),** 100 sheets

21065 **Pierce Background Eliminator Kit,** for eliminating background from X-ray film

46640 **SuperSignal Western Blot Enhancer,** 25 blots

84785 **SuperSignal Molecular Weight Protein Ladder,** 250µL

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer's exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).

There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.

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