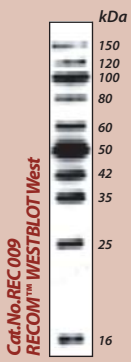
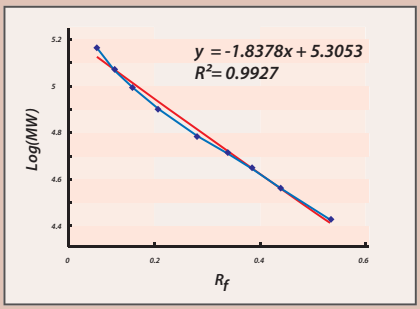
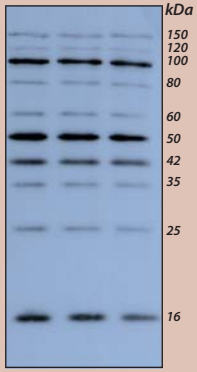
RECOM™ Pre-stained Protein Markers WESTBLOT Range

WESTBLOT Range Markers



Cat.No.REC 009
RECOM™ WESTBLOT Western Marker



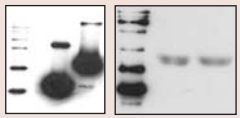


Analyzed in 10% gradient gel 10x10cm², 0.75mm dimension, Western by anti-mouse IgG-HRP (Sigma 1:4,000)

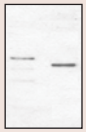
NOTE: This typical calibration curve cannot be used to derive laboratory test results. Each laboratory must prepare its own calibration curve.

Case Studies

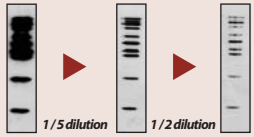
1. Uneven transfer, Loading error.



2. Secondary antibody may have problems. Marker has been over diluted.



3. Signal is too high, no distinct bands can be determined : Dilute 1/5 to 1/1,000 with 1x sample buffer, Do not reduce loading volume.



Real Biotech Corporation

www.real-biotech.com

RECOM™ Pre-stained Protein Markers WESTBLOT Range

Store at -20°C

■ Cat.No.REC 009
RECOM™ WESTBLOT Western Marker

Recommended Loading 5 ul/lane (250 µl)
Loading range may vary, check specifications

Applications
- Easy-to-use procedure
- Direct marker visualization on western blots (x-ray film, membranes, or on chemiluminescence imager)
- Accurate protein molecular weight estimation directly on western blots
- Optimize western transfer, antibody intactness, and detection system.

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Introduction

WESTBLOT western size marker is a brand-new, innovative true protein size marker. You can quickly and easily visualize discrete bands on your western blot only by a simple application of marker with your sample, You can get superior results, without further processing like adding extra reagents as described by many other suppliers. All the highly purified recombinant proteins are designed to bind immunoglobulins with high affinity in a denatured form, thus marker proteins directly bind to primary or secondary antibodies in your western blotting. You can use it in a chemiluminescent or chromogenic detection methods of your choice. It's compatible with most immunodetection methods, including horseradish peroxidase or alkaline phosphatase systems.

You'll get sharp, clear bands and precise, reproducible molecular weight estimation directly on western blot as shown on the figures.

WESTBLOT western size marker can also be used for optimizing your western transfer condition, verification of antibody conjugated peroxidase intactness, and controlling the detection system. For instance, size dependent uneven band densities of western size marker in western blotting will indicate inappropriate transfer of proteins to membrane and further adjustment of protein transfer condition. No marker bands in western blot may indicate functional problems in your detection system.

ADVANTAGES
- Easy-to-use procedure
- Direct marker visualization on western blots (x-ray film, membranes, or on chemiluminescen ceimager)
- Accurate protein molecular weight estimation directly on western blots
- Useful for optimizing western transfer, antibody intactness, and detection system.

SPECIFICATIONS

Contents :
250 µl of WESTBLOT western size marker in a ready-to-use format. (no need to boil before use).

Storage Buffer :
62.5mM Tris-HCl, pH 6.8, 1% β-mercaptoethanol, 35% glycerol, 1% SDS, and 0.01%bromophenol blue.

Sample Load :
5 µl of the marker solution is recommended on a 10x8cm mini-gel. Sufficient reagents are provided to perform 50 applications.

Although 5 µl is the recommended loading volume, you can load 0.01-10 µl depending on the binding affinity of your antibody species and the sensitivity of your detection system.Before applying in your samples, you must adjust appropriate loading quantity by serial dilution experiments. Load 5 µl of serially diluted marker (1x, 1/2x, 1/4x, 1/8x, and so on), and try western detection according to the same procedure as you do. Then determine best fitted loading volume to beautify your data.

Storage:
Upon arrival, store at -20°C. To avoid repeated freezing and thawing, aliquot in small volumes and store. This product is guaranteed for one year from date of purchase when properly handled and stored.

USER DIRECTIONS

Before you start , thaw the product at room temperature, and mix solution by brief vortexing.

Loading & Running
1. The WESTBLOT western size marker is provided in a ready-to-use format. There is no need to heat or reduce the standard prior to loading.
2. Load 5 µl of the WESTBLOT western size marker onto an appropriate SDS-PAGE gel.
3. Load your samples.
4. Run the samples using the desired buffer system.

Blotting & Detection
1. Transfer the proteins to a blotting membrane as described elsewhere.
2. Perform the blocking, primary antibody incubation, and secondary antibody incubation steps with the blot using a method of choice.
3. Visualize the proteins using a chemiluminescent or colorimetric detection system. After detection, you should observe ten protein bands ofthe standard as shown on the previous page.

NOTE: The 100 kDa and 50 kDa bands are at double concentration for quick identification.

TROUBLE SHOOTING

1. Week or no signals :
Check your detection system is functional. Check western transfer is completed.

2. Highly dense or dispersed signals :
Decrease marker loading volume up to 1/10 to 1/1,000. Dilute with 1x sample buffer.

3. Unusual dense band appeared :
Uneven western transfer due to size difference, check western transfer condition.