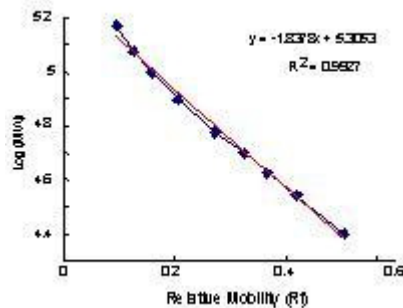
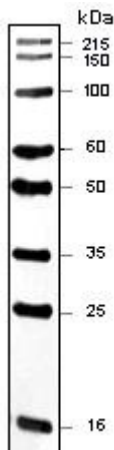


## Protocol

**Cat. No.:** REC091  
**Product Name:** RECOM WESTBLOT Wide Range Western Marker, 250ul  
**Cat. No.:** REC091S  
**Product Name:** RECOM WESTBLOT Wide Range Western Marker, Sample, 25ul

### Introduction

RECOM WESTBLOT Wide Range Western Marker is an innovative true size protein marker. Discrete bands could be quickly and easily visualized on your western blot only by a simple application of marker with your sample. Superior results could be obtained without further processing. The marker could be used in chemiluminescent or chromogenic detection. It's compatible with various immunodetection methods, such as horseradish peroxidase and alkaline phosphatase systems. Moreover, it can also be used for optimizing your western transfer condition, verification of antibody conjugated peroxidase intactness and controlling the detection system.



Analyzed in 7.5% Gradient Gel (10x10cm<sup>2</sup>, 0.75mm dimension). Western by anti-rabbit IgG-HRP(Sigma 1:3,000)

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

### Storage Buffer

62.5mM Tris-HCL, pH6.8, 1% β-mercaptoethanol, 35% glycerol, 1% SDS and 0.01% bromophenol blue.

### Recommended Loading Volume

1. Optimum 5ul/lane for a mini-gel. (10x8cm<sup>2</sup>, 0.75mm or 1mm thick).
2. Although 5ul is the recommended loading volume, you can load 0.01-10ul depending on the binding affinity of your antibody species and the sensitivity of your detection system.
3. Before applying in your samples, please adjust appropriate loading quantity by serial dilution experiments. Load 5ul of serially diluted marker (1X, 1/2X, 1/4X, 1/8X and so on). Then try western detection according to the same procedure. Then determine best fitted loading volume to beautify your data.



## **Storage Conditions**

RECOM WESTBLOT Wide Range Western Marker should be stored immediately upon receipt at  $-20^{\circ}\text{C}$  in a constant temperature freezer. RECOM WESTBLOT Wide Range Western Marker can be stored for up to 12 months without showing any deduction in performance and quality with proper storage.

## **User Directions**

### **1. Things Before Starting**

Thaw the marker at room temperature and mix solution by brief vortexing.

### **2. Loading & Running**

- 1) RECOM WESTBLOT Wide Range Western Marker is provided as ready-to-load format. No need to boil or reduce the standard before use.
- 2) Load 5ul of the marker on an appropriate SDS-PAGE gel.
- 3) Load your samples.
- 4) Run the samples using the desired buffer system.

### **3. Blotting & Detection**

- 1) Transfer the proteins to 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, observe ten protein bands of the standard as shown on the previous page.

## **Troubleshooting**

### **1. Weak or no signals.**

Make sure the detection system is functional. Make sure the western transfer is completed.

### **2. Highly dens 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. Make sure western transfer condition is correct.

## **Notes**

1. For best results, please aliquot into small volumes and store at  $-20^{\circ}\text{C}$ . Please prevent repeated freezing and thawing.
2. Repeated freezing and thawing, long-term storage and re-use of pipette tips may degrade proteins rapidly.
3. The marker is provided as ready-to-load format. No need to boil before use.