Ver. 1.3

Beacle, Inc.

Western Marker Neo

Western Protein ladder marker detected by 2nd antibody

User's Manual

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KYOTO JAPAN

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Cautions

1. Research use only. Do not use for medical purpose.

(1) Introduction

Western Marker Neo is the protein ladder marker for the exclusive use in western blot. The marker reacts with 2nd antibody used in western blot and detected by Enhanced Chemiluminescence (ECL) or other substrate.

●Features●

1. Pre-stained proteins included to monitor transfer efficiency
2. Shows good signals by various antibodies
3. Ready-to-use
4. 3 type marker with different molecular range availavle
5. Reasonable pricing

(2) Product lineup

Below table shows the lineup of the product. This manual is applied to all the products.

Product #	Product name	MW range	content
BCL-WMN-01	Western Marker Neo (low)	21-115 kDa	250 µL
BCL-WMN-11	Western Marker Neo (high)	43-190 kDa	250 µL
BCL-WMN-21	Western Marker Neo (wide)	21-190 kDa	250 µL

(3) Product information

Western Marker Neo contains antibody reactive marker proteins and pre-stained marker proteins in one product.

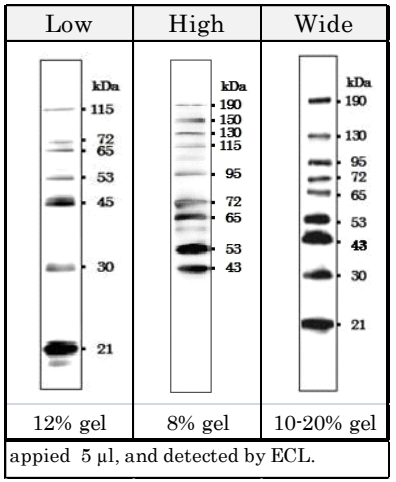
●Antibody-reactive marker proteins

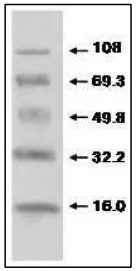
Western Marker Neo is provided with 3 MW ranges. The pattern of ladder signal can be seen in right Fig. Be sure that Low marker contains 45kDa, and High and Wide markers contain 43kDa protein.

Higher antibody concentration, which intensify marker signals, may cause additional bands (Low: 20, 46, 60 kDa, High: 60, 105 kDa, Wide: 20, 60, 115 kDa).

●Pre-stained marker proteins

All western marker products contain blue-colored proteins with MW range of 16 to 108 kD (see right Fig). This is for easier monitoring of electrophoresis status and transfer efficiency.





(4) Storage

store at -20℃

(5) Principle

Western Marker Neo is consisted of 11 recombinant proteins that have IgG binding domain. These proteins bind to IgG originated by essentially all mammal species. Thus regardless the origin and subtype, Western Marker Neo gives good signal. Western Marker Neo additionally contains 4 bluely pre-stained proteins (16-108 kDa). These proteins serve as a monitor protein during electrophoresis and transfer efficiency.

(6) How to use

＜General instruction＞

・The apply volume to gel is 2～5 µl/well.
・Apply directly to gels, no-pre-treatment is needed.

＜Detailed instruction＞

1. Apply to SDS-PAGE gel

Apply 2～5 µl of Western Marker Neo to gel wells along with your pr-treated sample, and separate proteins by electrophoresis.

【note 1】Change the apply volume depending on your experimental condition.
【note 2】During electrophoresis, blue line may be appear on the front.

2. Blotting to membranes

Take out the gel, set on the blotting apparatus, and transfer proteins to the membrane. Confirm that the blue-colored proteins can be seen in marker lane on the transferred membrane (refer to the figure in page 2)

【note 3】In some occasions blue color may be blotted on the front part in the transferred membrane, but the color will disappear in the following processes, and does not affect antibody reaction.

3. Blocking

Treat membranes with appropriate blocking solution.

4. Primary antibody reaction

Treat membrane with appropriate primary antibody solution. Primary antibody also binds to marker proteins during the reaction.

5. Secondary antibody reaction

Treat membrane with appropriate enzyme-labeled secondary antibody solution. Secondary antibody also binds to marker proteins directly or via the bound primary antibody during the reaction.

【note 4】As the labeled enzymes on secondary antibody, HRP (Horse Radish Peroxidase) or AP (Alkaline Phosphatase) can be used.

6. Detection

Detect secondary antibody-bound target and marker proteins by ECL reagents or by other ways. The marker proteins generate signals as does the target proteins, and are detected at the same time as is target protein.

【note 5】Principally, the marker proteins can be detected by colorimetric, fluorimetric methods. With ECL method, detection by X ray film other than CCD camera method is also possible.

7. Identification of marker proteins

Signal intensities of marker proteins are designed to be different each other. For example, 21 kDa protein shows strong signal, 30 kDa protein weak, and 190 kDa protein strong. The bands of 65 and 72 kDa proteins are in close proximity. These are good marks for identifying MW.

【note 6】Higher antibody concentration, which intensify marker signal, may cause additional band (refer to (3) Product Information for detail).
【note 7】The signal strength of marker is dependent on the origin (animal species) of antibody.

(7) Trouble shooting

Trouble	Cause and Measures
Too weak marker signal	1. Marker volume not enough. Increase volume.
	2. Low antibody concentration. Increase concentration.
	3. Transfer not enough. Increase transfer current or time.
	4. Too much transfer time and current. With nitrocellulose membrane, proteins go through the membrane. Decrease transfer current or time, or change membrane to PVDF.
Too strong marker signal	5. Marker volume too much. Decrease volume.
	6. High antibody concentration. Decrease concentration.
	7. Too long incubation time. Shorten the incubation time.
Fall out a part of band signal	8. In ECL, too strong signal is suppressed. Decrease the marker volume, or reaction time.
High background	9. High antibody concentration or long incubation. Decrease concentration or incubation time.
	10. Not enough blocking. Increase the blocking time or other conditions to increase blocking, or change the species of blocking agent.
	11. Not enough Washing. Increase the number or time of washing.

(8) Contact information

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