

EzProtein Ladder WB

Instruction Manual

Aug 6, 2025 5th edition

1. Precautions for safe use of this product

To use this product safely, please read this instruction manual carefully first. Please refrain from operating the product until you fully understand the contents of this instruction manual. This instruction manual describes only how to use this product for the specified purpose. Please refrain from using the product for purposes or in ways not described in this instruction manual. If you use the product for purposes or in ways not described in this instruction manual, you are solely responsible for all necessary safety measures and unforeseen circumstances. Also, please carefully read and understand the instruction manuals of any devices you will be using at the same time.

2. Purpose of use

This product is a molecular weight standard for Western blotting consisting of 4 colored bands and 10 bands (unstained) ranging from 15 to 200 kDa. The unstained bands have an IgG binding domain and bind to the primary and secondary antibodies in the antibody reaction of Western blotting, so they can be detected by chemiluminescence, fluorescence, or color development using various labeling enzyme substrates and Western blotting detection reagents. (Because the unstained bands are minute, they cannot be detected by CBB staining of the gel after electrophoresis. They cannot be used as molecular weight standards for electrophoresis.)

3. Product Configuration

Name	Volume	Quantity	Storage
EzProtein Ladder WB	0.25 mL/ tube	1本	-20°C

4. Composition

Name	Composition
EzProtein Ladder WB	Colored: 10, 25, 45, 70kDa Uncolored: 200, 150, 100, 80, 60, 50, 40, 30, 20, 15kDa SDS, glycerin, bromophenol blue (BPB), buffer solution

This Product does not contain any poisonous or deleterious substances under the Poisonous and Deleterious Substances Control Law, or any substances subject to notification that exceed the exemption amounts stipulated under the Industrial Safety and Health Law or the PRTR Law. For details, please download the SDS for this product from the ATTO website (<https://www.atto.co.jp/>).

5. Storage

- This product should be stored frozen (-20 °C or below). If it is unopened, it is stable until the expiration date. The expiration date is printed on the outer packaging.
- After dissolving this product, dispense into appropriate amounts and store frozen (-20 °C or below). Avoid freezing and thawing during use.

- This product is stable for approximately 3 months when refrigerated and for 12 months at -20 °C or below.

6. Disposal method

- Dispose of each reagent in accordance with the disposal method set out by your institution.

7. Other items required besides this product

- Electrophoresis gel
- Electrophoresis reagents (sample buffer, electrode solution, etc.)
- Electrophoresis equipment
- Small centrifuge

8. Precautions for use

- This product will be delivered refrigerated/frozen. Please store in a freezer (-20 °C or below) immediately after delivery.
- When dissolving this product, avoid heating or vigorous mixing and be careful not to create bubbles.
- After dissolving this product, mix well, dispense into appropriate amounts, and store in a freezer (-20 °C or below). Avoid freezing and thawing before use.
- This product can be stored stably for approximately 3 months in the refrigerator and for approximately 12 months at -20 °C or below.
- This product is a molecular weight standard for Western blotting. It cannot be used in experiments that do not involve antibody reactions. It cannot be used as a molecular weight standard for electrophoresis.
- It has been cross-confirmed with IgG from humans, horses, cows, pigs, rats, mice, rabbits, goats, sheep, hamsters, guinea pigs, etc. Please note that it does not cross-react with IgM.

2. How to use

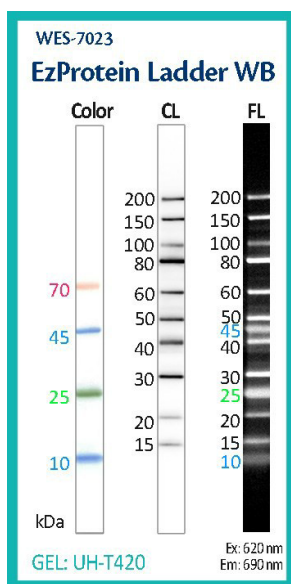
- Dissolve **EzProtein Ladder WB** completely at room temperature.
*Do not heat when dissolving.
- Mix thoroughly by tapping or inverting, taking care not to create bubbles.
*Please note that vigorous mixing with a vortex may cause the protein to break down.
- Spin down using a small centrifuge.
- When using mini gel electrophoresis, use 2.5-5μL per lane.
- After using it, promptly dispense and freeze. If you plan to use it again on the same day, store it on ice or in the refrigerator.
*After use, mix well, dispense into appropriate amounts, and store frozen (-20 °C or below). Avoid freezing and thawing.

6. Run the gel until the dye (BPB), which indicates the electrophoretic front, reaches 5 to 10 mm above the bottom of the gel.
7. After electrophoresis, transfer the proteins from the gel to a PVDF membrane or similar.
*If detecting by fluorescence, use a low-fluorescence PVDF membrane.
8. Block the membrane after transferring in the usual way, then react with the antibody.

10. Please detect according to the antibody label.

* If the antibody is HRP labeled, it can be detected by chemiluminescence or color development due to the HRP substrate. If it is fluorescently labeled, it can be detected by a combination of excitation light and filters appropriate for the label.

* Please note that if the excitation light is red, the 45, 25, and 10 kDa bands of the colored marker will be detected simultaneously, and if it is blue/green, the 70 kDa band will also be detected.



The separation pattern and molecular weight of **EzProtein Ladder WB**

Gel	u-PAGE H 4~20% (UH-T420) (4~20% polyacrylamide)
Marker	2.5 µL/Lane (EzProtein Ladder WB)
Running buffer	EzRun (Tris-Glycine-SDS)
Electrophoresis	300V constant voltage, 33 minutes
Detection	Left: Membrane after transfer detected with a scanner Center: After reaction with HRP-labeled secondary antibody, detected with luminol-based luminescence reagent EzWestLumi One Right: After reaction with Alexa647-labeled secondary antibody, detected with red excitation light (Ex: 620nm), filter (BPF690)

*Even if the experimental procedure is the same protocol, slight differences in technique can greatly affect the results. Tips and tricks are also important for obtaining optimal results. Please read the various "experiment tips" that are available for download from the ATTO website.

<https://www.atto.co.jp/>

