WSE -7025

EzStandard LMW Instruction Manual

August 27, 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 marker (unstained) for electrophoresis consisting of six proteins in the low molecular weight range (29 kDa or less). It can be visualized by various staining methods (silver staining, CBB staining, etc.).

3. Product configuration

Name	Volume	Quantity	Storage
Peptide stock (20x conc.)	0.1 mL	1	-20℃ or below
Sample buffer (2x conc.)	10 mL	1	-20℃ or below
Reducing agent (DTT)	150 mg	1	-20℃ or below

4. Composition

Name	Main component
Peptide stock (20x conc.)	buffer solution
Sample buffer (2x conc.)	Detergent, buffer solution, Coomassie brilliant blue (CBB)
Reducing agent (DTT)	Dithiothreitol

This product contains substances subject to notification that exceed the exemption quantities specified under the PRTR Act, the Poisonous and Deleterious Substances Control Act, and the Industrial Safety and Health Act. For details, please download and refer to 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.
- **Reducing agent (DTT)** should be stored frozen (−20 °C or below) even after dissolving in distilled water. After dissolving it, dispense into appropriate amounts and store frozen (–20 °C or below). Avoid freezing and thawing during use.
- Prepared **EzStandard LMW** is stable for approximately 6 months when refrigerated (2-10 °C). Avoid freezing and thawing during use.

6. Disposal method

- Dispose of each reagent in accordance with the disposal method of your affiliated institution.

7. Items required other than this product

- Block incubator (heating device)
- Microcentrifuge tube

8. Precautions for use

- This product will be delivered frozen. Please store in a freezer (–20 °C or below) immediately after delivery.
- **Reducing agent (DTT)** should be stored frozen (−20 °C or below) even after dissolving in distilled water. After dissolving it, dispense into appropriate amounts and store frozen (–20 °C or below). Avoid freezing and thawing before use (up to 5 times).
- Prepared **EzStandard LMW** is stable for approximately 6 months when refrigerated (2-10 °C). Avoid freezing and thawing during use.
- **EzStandard LMW** is a molecular weight marker for low molecular weight range. It is suitable for separation on high-concentration acrylamide gels such as Tricine PAGE.
- For visualization of **EzStandard LMW**, the staining method with **EzStain AQua** using a microwave is recommended. For detection with other staining methods, fix the gels before staining.

9. How to use

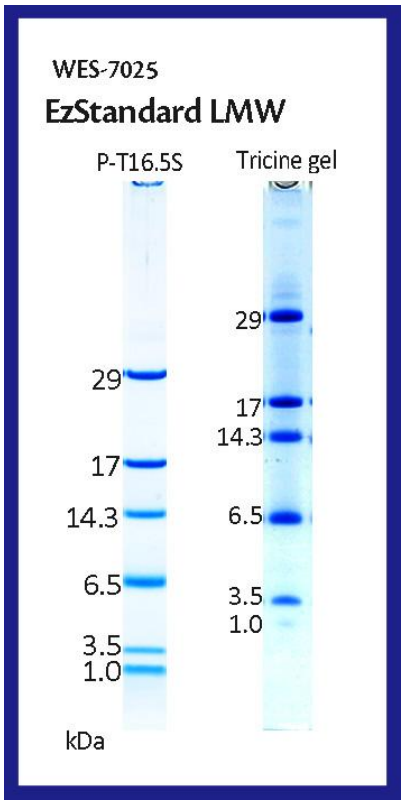
1. Dissolve **Reducing agent (DTT)** in 0.5 mL of distilled water. After dissolving, cool on ice. If it is difficult to dissolve, warm to 40-60°C. Store the prepared solution frozen (–20 °C or below).

   * After dissolving, dispense Reducing agent (DTT) into appropriate amounts and store frozen (–20 °C or below). Avoid freezing and thawing during use (up to 5 times).

2. Dissolve **Sample buffer (2x conc.)** completely at room temperature (15-30 °C).

   *Sample buffer (2x conc.) can be stored in the refrigerator (2-10°C) for a short period (several months). It can also be used to prepare each sample in the same way as the markers described below.

3. Mix 5 µL of **Peptide Stock (20x conc.)** with 50 µL of **Sample buffer (2x conc.)**.
4. Add 40 µL of distilled water to the solution from Step 3 and mix.
5. Add 5 µL of **Reducing agent (DTT)** to the mixture from step 4 and mix.
6. Incubate the mixture from Step 5 at 70 °C for 5–10 minutes.
7. Store the prepared marker at 2–10 °C. For electrophoresis, use 2–5 µL per lane.
8. Run the gel until the dye (CBB), which indicates the electrophoretic front, reaches 5–10 mm above the bottom of the gel.

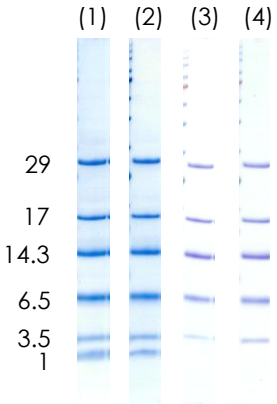


Protein	molecular weight	concentration ng/μL
Carbonic Anhydrase	29 kDa	182
Myoglobin	17 kDa	136
Lysozyme	14 kDa	182
Aprotinin	6.5 kDa	182
Insulin Chain B Oxidized	3.5 kDa	90
Bradykinin	1 kDa	90

Gel: (Left) P-T16.5S (**p-PAGEL**)
(Right) 16.5% Tricine gel (Shagger method)
Standard: 4 μL/lane
Running buffer: **EzRun T**
Running condition: (Left)175V constant voltage, 65 min
(Right)150V constant voltage, 120 min
Staining: **EzStain AQua** (Microwave method)

10. Others

The separation pattern and molecular weight of **EzStandard LMW.**



Notes on staining methods

The figure on the left shows the difference in detection sensitivity depending on the staining method. Results of **EzStandard LMW** separated on **p-PAGEL** and detected by the following methods: (1) **EzStain AQua**（using a microwave）,(2) **EzStain AQua**, (3) CBB staining after fixing the gel in 50% methanol / 10% acetic acid for 1 hour, (4) CBB staining. Using **EzStain AQua**, bands of 3.5 kDa and 1.0 kDa can be clearly detected. Note that prolonged fixation may reduce detection sensitivity (compare methods (3) and (4). To achieve sensitive detection of low-molecular-weight proteins, staining with **EzStain AQua** is recommended. In this case, use the microwave method to prevent diffusion of low-molecular-weight proteins. For homemade CBB staining solution, fix the gel in 50% methanol / 10% acetic acid for 20–30 minutes before staining with CBB.

ATTO CORPORATION
3-2-2 Motoasakusa, Taito-ku, Tokyo 111-0041, JAPAN
https://www.attoeng.com/
Contact