WSE-7057

EzRun BlueNative Instruction Manual

September 18, 2025 4th 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 electrode buffer for blue native PAGE using SDS-free Tris-Glycine polyacrylamide precast gels and homemade gels (Laemmli compliant).

3.Product configuration

Name	Volume	Quantity
EzRun BlueNative	500mL	1
BlueNative Buffer Additive	25mL	1

4.Composition

Name	Main components
EzRun BlueNative	Tris buffer
BlueNative Buffer Additive	Coomassie Brilliant Blue

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

- Store **EzRun BlueNative** and **BlueNative Buffer Additive** at room temperature (15–30°C), protected from direct sunlight. Unopened products are stable until the expiration date.
- Solutions prepared by diluting **EzRun BlueNative** should be stored tightly closed at room temperature (15–30°C), protected from direct sunlight.
- Electrophoresis buffer that has been used once cannot be reused.

6.Disposal method

- Dispose of each reagent in accordance with the disposal method of your affiliated institution.
- Bottle Material Body and lid : Polypropylene

7.Items required other than this product

- Magnetic stirrer
- Stirrer bar
- Beaker
- Graduated cylinder
- Medium bottles or other containers
- Distilled water
- Tris-Glycine polyacrylamide gel (recommended: **u-PAGEL H**)
- Filter paper (e.g., **CB-09A**)
- Blotting membrane (e.g., **WSE-4051**)
- Blotting buffer (recommended: **WSE-7210 EzFastBlot HMW**)
- Electrophoresis tank: (recommended: **AE-6530**, **WSE-1165 Mini-Slab**, etc.)
- Blotting apparatus: (recommended: **WSE-4025 HorizBlot 2M**, etc.)
- Power supply: (recommended: **WSE-3100 Power Station Ghibli I**)

8.Precations for use

- **EzRun BlueNative** is a 10x stock solution. Dilute according to the instructions before use.
- **BlueNative Buffer Additive** is a 100x stock solution.
- **BlueNative Buffer Additive** may precipitate. Mix thoroughly by inversion before use.
- **EzRun BlueNative** does not contain preservatives. After opening, handle with care to avoid contamination by microorganisms.
- This product is intended for native PAGE and cannot be used for SDS-PAGE.
- This product can be used with Tris-Glycine gels that do not contain SDS. It is not compatible with Bis-Tris gels or Tris-Acetate gels.

9.How to use

A. Preparation

Dilute **EzRun BlueNative** 10 times with distilled water.
To prepare 500 mL of electrode buffer, add 50mL of **EzRun BlueNative** to 450mL of distilled water and mix thoroughly.

B. Electrophoresis

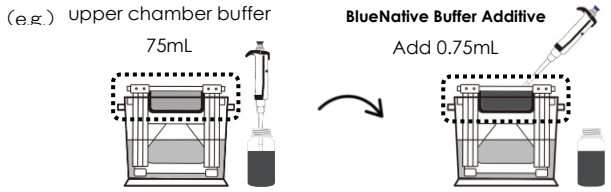
Note: When using this product, the procedure differs from the instructions provided with the device. Please follow the steps below.

1. Set the gel in the electrophoresis tank.
2. Add an appropriate volume of the diluted **EzRun BlueNative** to the lower chamber (anode side).
   * The required volume of buffer depends on the tank. Please refer to the table on the next page.

3. Add an appropriate volume of the diluted EzRun BlueNative to the upper chamber (cathode side).
   * The required volume of buffer depends on the tank. Please refer to the table below.
   *Add BlueNative Buffer Additive to the upper chamber buffer after sample application.

Gel size	apparatus	Required Volume of Buffer	Required Volume of **BlueNative Buffer Additive**
Mini	**AE-6530** **WSE-1150**	Cathode: 75 mL Anode: 250 mL	Cathode: 0.75mL
	WSE-1165	Cathode: 250 mL Anode: 250 mL	Cathode: 2.5 mL
Compact	**WSE-1010** **WSE-1025**	Cathode: 120 mL Anode: 70 mL	Cathode: 1.2 mL
	WSE-1030 **WSE-1040**	Cathode: 135 mL Anode: 110 mL	Cathode: 1.35 mL
Wide	**WSE-1170**	Cathode: 400 mL Anode: 400 mL	Cathode: 4.0 mL

4. Apply the samples.
5. According to the table above, add 1/100 of the volume of **BlueNative Buffer Additive** (relative to the upper chamber buffer) to the upper chamber buffer (cathode side).



6. Connect the electrophoresis tank to the power supply and perform electrophoresis.
   For one mini-size gel, electrophoresis can be performed at 20 mA constant current for 70–80 minutes, or at 150 V constant voltage for 80–90 minutes.
   - If using **PageRunAce (WSE-1150)**: run in STD mode for 70–80 minutes.
   - If using **Compact PAGE Ace (WSE-1010/25)**: run in STD mode for 20–30 minutes.
   - If using **Compact PAGE Neo (WSE-1030/40)**: run at 150 V constant voltage for 35–40 minutes.
   - For wide-size gels: run at 30 mA constant current or 150 V constant voltage.

   *Follow the instructions provided with each device for proper use.

7. When the dye front (CBB line) reaches the bottom of the glass plate, turn off the power to stop electrophoresis.
   *If you plan to perform blotting, proceed directly to C. Blotting without destaining.
8. After electrophoresis, immerse the gel in destaining solution (50% methanol, 12.5% acetic acid) and gently agitate until the background becomes clear.
9. Once the background is clear, immerse the gel in a sufficient amount of distilled water and gently agitate. Then proceed with gel imaging or other analyses

For re-staining

Gels that have been destained can be re-stained. After completing step B.9, immerse the gel in a staining solution or fixing solution, then perform staining and destaining as required. Follow the instructions provided with the staining reagent you are using. CBB or silver staining can be used.

C Blotting

*Semidry using **EzFastBlot HMW (WSE-7210)** or conventional wet transfer is recommended.
*Prepare the following buffers before starting blotting
*Pre-wet the PVDF membrane with methanol, and then equilibrate it in the transfer buffer prepared in step (2) below.

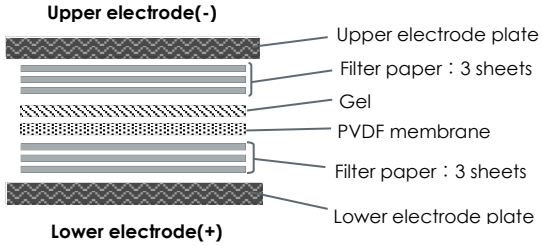
(1) Gel Pre-treatment Solution（50 mL per mini-size gel）

Composition**: EzFastBlot HMW** + 0.1% SDS + 10% methanol
Preparation: To 10 mL of **EzFastBlot HMW**, add 50 mg of SDS and 5 mL of methanol, then adjust the volume to 50 mL with distilled water.

(2) Transfer Buffer（200 mL per mini-size gel）

Composition: **EzFastBlot HMW** + 10% methanol
Preparation: To 40 mL of **EzFastBlot HMW**, and 20 mL of methanol, then adjust the volume to 200 mL with distilled water.

1. Immerse the gel (after electrophoresis) in the Gel Pre-treatment Solution prepared in step (1), and gently agitate to equilibrate for approximately 10 minutes.
2. Assemble the blotting sandwich in the following order, referring to the diagram below:.



   1) Drop a few milliliters of Transfer Buffer onto the lower electrode plate to pre-wet it.
   2) Place 3 sheets of filter paper soaked in the Transfer Buffer on the lower electrode plate.
   3) Place the PVDF membrane on top of the filter paper.
   4) Drop a few milliliters of Transfer Buffer onto top of the PVDF membrane.
   5) Carefully place the gel on the PVDF membrane, ensuring that no air bubbles are trapped between the gel and the membrane.
   6) Place 3 sheets of filter paper soaked in Transfer Buffer on top of the gel.
   7) Using a blotting roller or similar tool, gently remove any excess buffer or air bubbles between the gel and the PVDF membrane, ensuring tight contact.
   8) Gently lower the upper electrode plate onto the stacked filter paper.

3. Connect the blotting apparatus and power supply using the leads. Follow the instructions provided with each device for proper operation.
4. Apply a constant voltage of 6–12 V for 60–120 minutes.
5. After transfer, immerse the membrane in methanol and remove CBB by replacing the solution 2–3 times (within 30 seconds). Then rinse the membrane with distilled water or TBS-T.
6. Perform blocking, antibody reactions, and detection as usual.
   *When using transfer buffers other than **EzFastBlot HMW**, follow the same blotting procedure described above.

Note on Western Blotting with BlueNative PAGE

In Western blotting using BlueNative PAGE, the isoelectric point of proteins can have a significant effect. To ensure that most proteins are negatively charged, a transfer solution with high pH is used.Additionally, in BlueNative PAGE, Coomassie Brilliant Blue (CBB) binds to proteins, providing negative charge without dissociating complexes. However, because of the strong affinity of CBB, it may remain bound to proteins, causing highly negatively charged proteins to pass through the membrane during transfer. As a result, bands on the membrane may be difficult to detect.
In such cases, detection of bands may be improved by:

- Increasing the amount of sample applied,
- Staining the membrane with a high-sensitivity CBB dye
- Adding 10% methanol to the transfer buffer.

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