WSE- 7067

EzBlueNative Additive

Operating instructions

Aug 13, 2025 2nd 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 CBB additive for the cathode running buffer used in Blue Native PAGE with gels (SDS-free) prepared using gel buffers such as Tris, Tris/Gly, Tris/MOPS, or Bis-Tris based.

3. Product configuration

Name	Volume	Quantity
EzBlueNative Additive	25 mL	1 bottle

4. composition

Name	Main component
EzBlueNative 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

- **EzBlueNative Additive** at room temperature (15–30 °C), away from direct sunlight. Unopened, it is stable until the expiration date (shown on the label).
- Once used, the running buffer cannot be reused.

6. Disposal method

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

7. Items required other than this product

- Magnetic stirrer, Stir bar
- Beaker, Graduated cylinder
- Medium bottle or other containers, Distilled water
- Polyacrylamide gel
  Tris, Tris/Gly, Gels made with Tris/MOPS or Bis-Tris gel buffer (without SDS),
  [Recommended] **u-PAGEL H** (Tris/Gly gel)
- Running buffer
  Refer to Table 1 and select an appropriate solution according to the gel buffer.
- Electrophoresis sample preparation
  WSE-7011 EzApply Native
- Filter paper (**CB-09A,** etc.)
- Blotting membrane (**WSE-4051,** etc.)
- Blotting buffer
  [Recommended] **WSE-7210 EzFastBlot HMW**
- Electrophoresis tank:
  AE-6530 Lapidus Mini Slab Electrophoresis Tank
  WSE-1165 Lapidus Mini Slab Electrophoresis Tank etc.
- Blotting equipment:
  [Recommended] **WSE-4025 Horizon Blot 2M** etc.
- Power supply:
  [Recommended] **WSE-3100 Power Station Ghibli I**

8. Precautions for use

- **EzBlueNative additive** is a 100x stock solution.
- **EzBlueNative Additive** may cause precipitation of its ingredients. Mix by inversion before use.
- This product is a reagent for Native PAGE and cannot be used for SDS-PAGE.

Table 1: Gel and running buffer combinations

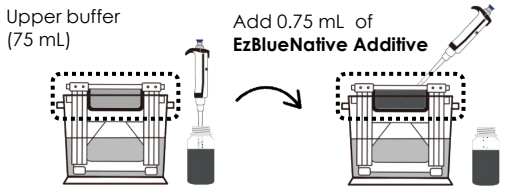
Gel	Anode Buffer	Cathode Buffer	
Tris/Gly -based gel **u-PAGEL**, **e-PAGEL HR**, **e-PAGEL**, **c-PAGEL Neo,** Tris -based homemade gel (125 mM Tris (pH 6.8)/375 mM Tris (pH 8.8)	**WSE-7066 EzRun MOPS Non- SDS**	**WSE-7066 EzRun MOPS Non- SDS**	**EzBlueNative Addi tive** (Add 1/100 volume of cathode buffer immediately before electrophoresis)
	WSE-7055 EzRun T.G. (25 mM Tris/192 mM Glycine)	**WSE-7055 EzRun T.G.** (25 mM Tris/192 mM Glycine)	
Bis-Tris gel 0.5M 6-Aminocaproic acid/50 mM Bis-Tris (pH 7.0)	50 mM Bis -Tris/pH7.0	50 mM Tricine/15 mM Bis-Tris/pH 7.0	
Imidazole -based gel 0.5M 6-Aminocaproic acid/25 mM imidazole (pH 7.0)	25 mM imidazole/pH 7.0	50 mM Tricine/7.5 mM imidaz -ole/pH7.0	

9. How to use

A. Electrophoresis

*When using this product, please note that the operating procedure differs from that described in the instruction manual supplied with the apparatus.

1. Place the gel in the electrophoresis tank.
2. Add an appropriate amount of electrophoresis buffer to the lower chamber (anode side) and upper chamber (cathode side) of the electrophoresis tank (see Table 1 on the previous page).
   * **EzBlueNative additive** is added to the upper buffer after sample application.
3. Apply the sample.
4. Add 1/100 volume of **EzBlueNative Additive** to the upper chamber (cathode side) running buffer.



5. Connect the electrophoresis tank to a power source and run electrophoresis at a constant current of 20 mA or a constant voltage of 150 V per mini-size gel. This takes 70-80 minutes at a constant current of 20 mA, and 80-90 minutes at a constant voltage of 150 V.

WSE-1150 **PagerunAce:** 『STD』 mode
WSE-1030/40 **CompactPAGE Neo:** 20 mA/gel constant current or 150 V constant voltage (older models: "STD" mode)
Wide size gel: 30 mA/gel constant current or 150 V constant voltage

6. When the electrophoresis front (CBB line) reaches the bottom of the glass, turn off the power and end electrophoresis.
7. After electrophoresis, immerse the gel in destaining solution (50% methanol, 12.5% acetic acid), changing the destaining solution as necessary and shaking.
   *If proceeding to blotting, proceed to step B, blotting, without destaining.
8. Once the background becomes transparent, immerse the gel in a sufficient amount of distilled water and shake. Then photograph the gel.
   *After destaining, the gel can be re-stained with CBB staining, silver staining, etc.

B. Blotting

*We recommend semi-dry transfer using **WSE-7210 EzFastBlot HMW** or general wet transfer methods.

*Before blotting, prepare the following buffers.

① Gel pretreatment solution (50 mL per mini - size gel)

EzFastBlot HMW + 0.1% SDS, 10% methanol

Add 50 mg of SDS and 5 mL of methanol to 10 mL of **EzFastBlot HMW** and then add distilled water to make a 50 mL solution.

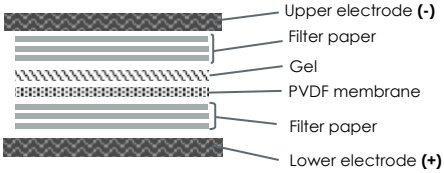
② Transfer buffer (200 ml per mini -size gel)

EzFastBlot HMW + 10% methanol

Add 20 mL of methanol to 40 mL of **EzFastBlot HMW** and make up to 200 mL with distilled water.

*Preliminarily hydrophilize the PVDF membrane with methanol, then equilibrate the PVDF membrane with the transfer buffer prepared in step ② above.

1. After electrophoresis, immerse the gel in the Gel pretreatment solution prepared in step ① above, shake it, and allow it to equilibrate for approximately 10 minutes.
2. Referring to the diagram below, stack the filter paper, membrane, and gel in the correct order.



(1) Drop a few mL of the transfer buffer onto the lower electrode plate to wet the electrode plates beforehand.

(2) Place three sheets of filter paper soaked in the transfer buffer (step ②) on top of the lower electrode plate.

(3) Place the PVDF membrane on top of the filter paper.

(4) Drop a few mL of the transfer buffer onto the PVDF membrane.

(5) Place the gel on top of the PVDF membrane, taking care not to trap air bubbles between the PVDF membrane and the gel.

(6) Place three sheets of filter paper soaked in the transfer buffer (mentioned in ②) on top of each other.

(7) Using a blotting roller or similar tool, carefully remove any excess buffer or air bubbles between the gel and the PVDF membrane, ensuring a tight bond between the gel and the membrane.

(8) Gently place the upper electrode plate onto the stack of filter papers.

3. Connect the blotting device to the power supply with lead wires. Follow the instructions in the attached manual for each device.
4. Apply a constant voltage of 6 to 12V for 60 to 120 minutes.
5. After transferring, immerse the membrane in methanol and change the solution 2 to 3 times to remove CBB from the membrane as needed (within 30 seconds). Then rinse with distilled water or TBS - T.
6. Proceed with blocking, antibody reaction, and detection as usual.

   *When using a transfer buffer other than **EzFastBlot HMW**, please perform blotting using the same procedure as above.

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