WSE-7135

# EzPreStain DNA&RNA Instruction Manual

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## 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 fluorescent reagent used for staining DNA and RNA in agarose gels during electrophoresis when dissolved in an agarose gel solution or electrophoresis buffer. It can also be used as a post-electrophoresis staining solution, in which case it enables the staining of DNA and RNA in both agarose and polyacrylamide gels. The stained gel can be excited with cyan or blue LEDs, UV light (450-480 nm) and observed by fluorescence of 500-600 nm (500-550 nm LP filter is suitable). This fluorescent staining reagent is safer than ethidium bromide and has high detection sensitivity.

## 3.Product configuration

| Name | Volume | Quantity |
|---|---|---|
| **EzPreStain DNA&RNA** | 0.5 mL | 1 |

## 4.Composition

| Name | Main components |
|---|---|
| **EzPreStain DNA&RNA** | DMSO |

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 this product in a dark place and in a freezer (–20 °C or below). If unopened, it is stable up to the expiration date. The expiration date is printed on the outer box and reagent bottle.

## 6.Disposal method

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

## 7.Items required other than this product

- Fluorescent gel imaging device (cyan or blue LED, UV)
- Electrophoresis equipment
- Agarose gel or polyacrylamide gel
- Electrode buffer solution, etc.

## 8.Precations for use

- Store this product in a dark place and in a freezer (–20 °C or below).
- This product may have mutagenic potential; therefore, please wear rubber gloves and avoid direct contact with the skin.
- If the undiluted solution comes into direct contact with a plastic container, the container may discolor or deform.
- If a glass container is used when adding this product to a buffer solution or staining a gel the fluorescent reagent will be adsorbed to the glass. Be sure to use a plastic container for staining.
- This product is supplied at a 10,000× concentration. Please dilute it with an agarose gel solution or a buffer such as TAE, TBE, or TE before use. When using it as a staining solution, dilute it with a buffer such as TAE, TBE, or TE.
- After staining, detect the gel by excising it with cyan or blue LEDs, UV (Ex: 250/370/482 nm; Em: 509 nm). Do not look directly at the irradiation device.
- Please note that this product cannot be added directly to the sample for detection.
- When using polyacrylamide gels, please note that the pre-staining method (adding this product to the electrode buffer) is not suitable for detection.

## 9.How to use

There are three methods for using **EzPreStain DNA&RNA**:

A. Adding it to the electrode buffer (pre-staining method)
B. Adding it to the agarose gel solution (pre-staining method)
C. Using it as a staining solution (post-staining method)

Each method is described below.

### A. Method for adding to the electrode buffer

**Note**: When using polyacrylamide gels, this method is not suitable for detection.

1. Prepare an agarose gel using an appropriate buffer (e.g., TAE or TBE) at a concentration suitable for the target molecular weight range.

2. Add **EzPreStain DNA&RNA** to the electrode buffer at 1/10,000 of the buffer volume.
   For example, add 25 µL of **EzPreStain DNA&RNA** to 250 mL of electrode buffer and mix well.
   * Use the same buffer as the gel buffer.
   * Wear rubber gloves and avoid direct contact with the skin.

3. Place the agarose gel prepared in step 1 into the electrophoresis tank.

4. Pour the electrode buffer prepared in step 2 into the tank.

5. Perform electrophoresis under light-shielded conditions.
   * When the electrode buffer containing **EzPreStain DNA&RNA** is added to the tank, the tank may appear slightly yellow. After electrophoresis, wash the tank promptly.

6. After electrophoresis, place the gel on an imaging system (cyan or blue LED, or UV) and capture the image.

**B. Method for adding to the agarose gel solution**

**Note**: Long electrophoresis times may affect staining, especially for low-molecular weight. If performing electrophoresis for more than 45 minutes, use method A.

1. Weigh agarose to achieve the desired concentration and add the appropriate volume of gel buffer (e.g., TAE or TBE).

2. Heat the mixture in a microwave or water bath until the agarose is completely dissolved.
   * The dissolved agarose gel solution is extremely hot and may boil over. Always wear heat-resistant gloves and handle with care.
   * Do not add **EzPreStain DNA&RNA** before heating.

3. Cool the agarose gel solution to approximately 60°C, then add **EzPreStain DNA&RNA** at 1/10,000 of the gel solution volume.
   For example, add 4 µL of **EzPreStain DNA&RNA** to 40 mL of gel solution and mix well.
   * Wear rubber gloves and avoid direct contact with the skin.

4. Pour the agarose gel solution containing **EzPreStain DNA&RNA** into a gel tray.
   Cover with aluminum foil or similar and allow it to solidify at room temperature for 30–60 minutes.
   * The agarose gel may appear slightly yellow. Prepared gels can be stored at 4°C for up to 3 days, protected from light.

5. Place the solidified gel into the electrophoresis tank and perform electrophoresis under light-shielded conditions.
   The recommended voltage is 4–10 V/cm (between the gel's anode and cathode).

6. For example, for a gel with a 6 cm distance between electrodes, apply 24–60 V/gel.
   * High voltage may affect staining. Do not exceed the recommended settings.

7. After electrophoresis, place the gel on an imaging system (cyan or blue LED, or UV) and capture the image

**C. Method for using as a staining solution**

1. Perform electrophoresis using an agarose gel or polyacrylamide gel appropriate for the target nucleic acid molecular weight, in an appropriate buffer (e.g., TAE or TBE).
   * Do not add **EzPreStain DNA&RNA** to the gel or electrode buffer used for running.

2. Prepare the staining solution by adding **EzPreStain DNA&RNA** to the same buffer used for electrophoresis at 1/10,000 of the buffer volume.
   For example, add 5 µL of **EzPreStain DNA&RNA** to 50 mL of buffer and mix well.
   * Use a plastic container when preparing the staining solution, as fluorescent reagents may adsorb to glass.

3. Immerse the gel in the staining solution prepared in step 2 and stain at room temperature for 10–30 minutes, protected from light.
   * Use a plastic container and cover with aluminum foil or similar to block light.
   * Staining time depends on gel thickness and DNA/RNA concentration. No destaining is required.
   * After staining, the solution can be stored at 4°C for approximately 1 week, protected from light.

4. Place the stained gel on an imaging system (cyan or blue LED, or UV) and capture the image.

**Imaging conditions:**

- Cyan LED excitation: 480–530 nm, filter: 500–550 LP
- Blue LED excitation: 440–500 nm, filter: 500–550 LP
- UV excitation: 260–370 nm, filter: 500–550 LP

* Peak excitation/emission: Ex: 250/370/482 nm, Em: 509 nm


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