AE-1310 EzStain Reverse

1. Safety Precautions

EzStain Reverse is a kit used for staining gels after electrophoresis. Do not use EzStain Reverse before you fully understand how to use the electrophoresis apparatus and power supply unit. This product should be used only for its intended use as described in the instruction manual.
As a pretreatment solution, 10% methanol is used. Follow the precautions on the label of the reagent (methanol).

2. Introduction

EzStain Reverse is a kit used for negative staining gels after electrophoresis of proteins. As the name of the product implies, EzStain Reverse whitens the background of gel while keeping the protein bands transparent.

3. Features

(1) Staining time of only 25 minutes
(2) Approximately 10 times highly sensitive than CBB stain and approximately one-third less sensitive than silver stain
(3) Easy to handle: All you need other than EzStain Reverse are only methanol and purified water, which will be used for diluting "R-1 Solution" and "R-2 Solution."

4. Components

EzStain Reverse is composed of "R-1 Solution" and "R-2 Solution." Each kit will stain 50 mini slab gels (approximately 90 x 80 x 1 mm).

Solution R-1 (Staining solution)/ Imidazole, sodium dodecyl sulfate (SDS)
Solution R-2 (Color developing solution)/ Zinc sulfate

5. Method of Disposal after Use

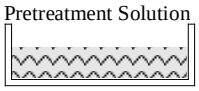
"Solution R-2" (color developing solution) contains zinc ion. After use, "Solution R-1" and "Solution R-2" should be mixed at a ratio of 1 : 1 as the original solutions, to form a precipitate. Collect the precipitate by filtration and dispose properly.
When disposing "Solution R-2" solely, without mixing with "Solution R-1," increase the pH of "Solution R-2" (color developing solution) to 8.5 or higher by adding calcium hydroxide or anhydrous sodium carbonate aqueous solution, to form a precipitate. Then collect the precipitate by filtration and dispose properly.
The components of this product, other than zinc ion, may be irritant to skin or mucosa. If any of the components is attached to skin or eye, wash immediately. If inflammation occurs, immediately consult a physician.

6. Storage

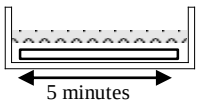
EzStain Reverse should be stored at room temperature, avoiding light. Unopened reagent is stable until mentioned expiration date.

7. Procedure

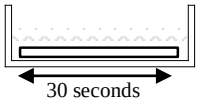
7.1 The method for staining of a polyacrylamide mini slab gel (90 × 80 × 1 mm) is explained below. The quantity of the reagents required for staining of a slab gel (130 × 135 × 1 mm) is twice as much of that for a mini slab gel.
7.2 Equipments and apparatus required: Graduated cylinder, beaker, tray for staining, shaker, waste bin
7.3 Purified water (200 mL) and methanol (10 mL) (not included in this kit)
7.4 Set-up Solution Procedure
(1) “Pretreatment Solution”: Add purified water to 10 mL of methanol to give a total volume of 100 mL.
(2) “Staining Solution”: Mix 10 mL of “Solution R-1” and 50 mL of purified water.
(3) “Color Developing Solution”: Mix 10 mL of “Solution R-2” and 50 mL of purified water.
7.5 Staining Procedure
(1) [Decantation of “Pretreatment Solution” to the tray]
Before electrophoresis, decant “Pretreatment Solution” to a tray that is several centimeters larger than the gel.



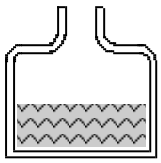
(2) [Soak of gel in “Pretreatment Solution”]
After electrophoresis, immediately soak the gel in “Pretreatment Solution,” and incubate it for 5 minutes with a level of agitation that is enough to make the gel is always left from the bottom of the tray. Then discard the solution.



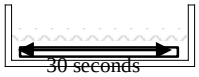
(3) [Washing of gel with 100 mL of purified water]
Pour 100 mL of purified water to the tray, and wash gels for approximately 30 seconds. Then discard the water.



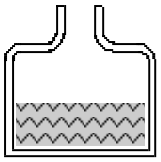
(4) [Soak of gel in “Staining Solution”]
Pour 60 mL of “Staining Solution” to the tray, and incubate the gel for 10 minutes (5% gel), 20 minutes (20% gel) or 15 minutes (5 - 20% gel).
Then discard the solution in the waste bin.

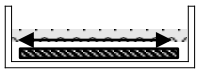


(5) [Washing gel with purified water]
Pour 100 mL of purified water to the tray, and wash the gel for approximately 30 seconds. Then discard the water.



(6) [Soak of gel in “Color Developing Solution”]
Pour 60 mL of “Color Developing Solution” to the tray and incubate it. Color develops within 1 - 3 minutes. When slightly light-colored electrophoresis bands are obtained, discard the solution in the waste bin in which “Staining Solution” was placed in (4) above.
Coloration is proceeding.

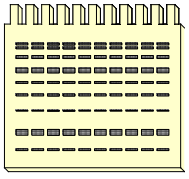




(7) [Washing gel with purified water]
Pour 100 mL of purified water to the tray, and wash the gel for 2 minutes. Then discard the water. Color development will be stopped.

(8)[Re-pouring of purified water]
Pour purified water again to the tray and incubate it for 5 minutes, to stop color development securely.

(9) Record the electrophoresis images just after staining.
When recording the gel images later, put the gel, with a small amount of purified water, in a plastic bag and close it tightly. This allows approximately 1-week storage.
(10) Supplementary note: The placement of a piece of black paper under the tray makes it easier to monitor the progression of color development described in (6).



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