AE-1360

EzStainSilver 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.
This product contains ingredients that may irritate the skin and mucous membranes. When handling, be sure to wear gloves or other protective gear. When using a pipette, never suck with your mouth. If the solution comes into contact with your skin or eyes, rinse immediately with tap water. If you experience any abnormalities, seek medical attention immediately..

2. Purpose of use

This product is a kit for silver staining proteins and DNA after polyacrylamide gel electrophoresis. It is capable of detecting several ng/band of protein and several tens of pg/band of DNA.

3. Product configuration

Name	Volume	Quantity
S-1 solution	50 mL	1
S-2 solution	50 mL	1
S-3 solution	50 mL	1
S-4 solution	50 mL	1

4.Composition

Name	Main component
S-1 solution	Sodium thiosulfate
S-2 solution	Silver nitrate
S-3 solution	Sodium hydroxide
S-4 solution	Formaldehyde, sodium thiosulfate

This product contains substances subject to notification that exceed the exemption quantities specified under the PRTR 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. Strage

- Store in a dark, refrigerated location (2-10°C).
- When stored unopened and refrigerated (2-10°C), it is stable up to the expiration date (2 years from the date of manufacture).
- The expiration date for this product is indicated on the label.

6. Disposal method

The main component of **S-2** solution is silver nitrate. Please collect the solution containing **S-2** in a separate container from other solutions.
When disposing of a solution containing silver ions, add hydrochloric acid to the container to precipitate the silver chloride before disposal (approximately 500 µL of 1M HCl per 100 mL of staining solution).
Dispose of each reagent in accordance with the disposal method of your affiliated institution.

- Bottle material Body : High density polyethylene (HDPE)
  Lid : Polypropylene (PP)
  Gasket : Thermoplastic elastomer (TPE)

7. Items required other than this product

- Measuring cylinder
- Micropipette
- Beakers
- Staining containers
- Shaker
- Container for collecting waste liquid
- Distilled water
- Methanol
- Acetic acid or Citric acid

8. Pre-preparation of each solution

- Prepare the following five solutions in advance. These solutions should be prepared immediately before use.

"Fixative solution": Distilled water: 40 mL + Methanol: 50 mL
+ Acetic acid: 1 0 mL + **S-1** solution: 1 mL

"Staining solution": Distilled water: 100 ml + **S-2** solution: 1 ml

"Developing solution":
Distilled water: 200 ml + **S-3** solution: 1 ml+ **S-4** solution: 1 mL

"Stop solution": Distilled water: 100 ml + Acetic acid: 1 ml
or Distilled water: 100 mL + Citric acid: 3 g

""DTT washing solution"": 30 % Methanol solution: 100 mL x 2
Prepare this if your sample was treated with DTT.

9. How to use

The following procedure is for staining a mini slab gel (90 mm x 80 mm, 1 mm thick).

①Pour 100 mL of fixative into a container, immerse the polyacrylamide gel after electrophoresis in the fixative, and shake for 10 minutes.
* Please prepare a container that is approximately 50% larger than the gel size.
*Shake quickly enough so that the gel does not stick to the bottom of the container.
* The processing time for this step is determined based on the thickness of the gel .
→ Gel thickness: 0.75 mm: 5 minutes
→ **Gel thickness: 1 mm : 10 minutes**
→ Gel thickness: 2 mm : 20 minutes

② Discard the fixative and wash the gel by adding 100 mL of distilled water and shaking for 10 minutes.

*If the sample was treated with DTT, use 100 mL of 30% methanol solution, which is the DTT washing solution.
*Shake vigorously until the gel sinks, then reduce the speed once it is submerged in the washing solution.
*The processing time for this step is also set depending on the thickness of the gel.

→ Gel thickness: 0.75 mm: 5 minutes
→ **Gel thickness: 1 mm : 10 minutes**
→ Gel thickness: 2 mm : 20 minutes

③ Repeat step ② again .

④ Add 100 mL of distilled water with or without DTT and shake for 10 minutes to wash the gel.

⑤ Discard the distilled water, add the entire amount of staining solution, and shake for 5 minutes.

* The processing time for this step will depend on the thickness of the gel.

→ Gel thickness: 0.75 mm : 3 minutes
→ **Gel thickness: 1 mm : 5 minutes**
→ Gel thickness: 2 mm : 10 minutes

⑥ Recover the staining solution, add 100 mL of distilled water, and shake for 30 seconds to wash the gel.

*Recover the staining solution separately from other solutions as silver waste.

⑦ Discard the distilled water, add 100 mL of developing solution, and shake for exactly 30 seconds.

⑧ Discard the first developing solution and add the remaining 100 mL of developing solution. Stop shaking before the desired staining occurs .

* Developing time varies depending on the sample, temperature, etc.
* The color development will continue even if you add the stop solution in the next step ⑨.
* The processing time for this step is also set depending on the thickness of the gel.

→ Gel thickness: 0.75 mm: 5 minutes
→ **Gel thickness: 1 mm : 10 minutes**
→ Gel thickness: 2 mm : 20 minutes

⑨ Discard the developing solution and immediately add the entire volume of stop solution and shake for 10 minutes.

⑩ Discard the stop solution, add 100 mL of distilled water, and shake for 5 minutes to wash .

⑪ Repeat the washing process again.

10. Additional information

Each processing time has been optimized. Be sure to operate according to the times specified in this instruction manual.

	Step	Solution	Time
①	Fixation	Fixative solution	10 minutes
②～④	Washing	Distilled water	10 minutes x 3 times
⑤	Staining	Staining solution	5 minutes
⑥	Washing	Distilled water	30 seconds
⑦	Pre-developing	Developing solution	30 seconds
⑧	Developing	Developing solution	Varies by sample
⑨	Stopping	Stop solution	10 minutes
⑩～⑪	Washing	Distilled water	5 minutes x 2 times

•When immersing the gel in a solution, make sure the gel is completely submerged in the solution..
•Silver stain is very sensitive so it may be affected by sample solution, process liquid and so on. Especially, color tone of background may be different from that of the surrounding of sample added lane due to DTT. When you wash gel, please refer to description about DTT.
•If staining takes two days, soak the gel in fixative on the first day and start by washing with distilled water the next day.
•It is possible to perform silver staining after CBB staining, but in this case, please make sure to completely destaining the CBB dye before starting the silver staining procedure.

11. Reference materials

Various documents can be downloaded from the ATTO website, so please take a look.
https://www.atto.co.jp/

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