

# AE-1360 *EzStain Silver* Instruction Manual

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## 1. Safety precaution

Before using this product, read this instruction manual thoroughly at first. Do not start the operation until you understand the contents of manual sufficiently. Also, this document describes only method used for specified purpose with this product. Do not use this product for any purpose or by any method other than described here. If you use this product for any purpose or by any method other than described in this manual, you will be held responsible for any necessary safety measures as operator.

This product includes components which might stimulate skin or mucosa. When it is used, take an action such as putting on gloves for protecting your body from it. When you use a pipette, do not pipette it by mouth. If this solution adheres to your skin or eye, wash it down with tap water immediately. When you feel sick or something strange, receive treatment from doctor immediately.

## 2. Application purpose

This product is a silver staining reagent used for protein or DNA in polyacrylamide gel after electrophoresis. It can detect several ng protein/band and several tens pg DNA/band.

## 3. Package

Product name	Volume	Package
S-1 solution	50 mL	1 bottle
S-2 solution	50 mL	1 bottle
S-3 solution	50 mL	1 bottle
S-4 solution	50 mL	1 bottle

## 4. Components

Product 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 includes notifiable materials exceeding to regulated amount for excluding decided by PRTR Law and Industrial Safety and Health Law. Please contact our sales department about its MSDS.

## 5. Storage

- Close the lid tightly and keep this product in a refrigerator (5-10°C) away from light.
- It is stable under the above condition until expiration date for use (2 years from production date).
- The storage period is described on the label.

## 6. Disposal method

- The main component of S-2 solution is silver nitrate, so that the solution needs to be collected as silver a heavy metal in container.
- If solution including silver ion is disposed, put hydrochloric acid in container to precipitate it as silver chloride and treat it as heavy metal. (1M HCl about 500uL/staining reagent 100mL)
- Follow the disposal method decided by the organization you belong to.
- Material of bottle

Main body: High density polyethylene

Lid: Polypropylene

Packing: Thermoplastic elastomer

## 7. Necessary things other than this product

- Graduated cylinder
- Micropipette
- Beaker
- Tray for staining
- Shaker
- Container for collecting waster fluid
- Purified water
- Methanol
- Acetic acid or Citric acid

## 8. Preparation of each solution

- Prepare the below 5 kinds of solution in advance. These are prepared at time of use.

「Fixative」:

Purified water: 40 mL + Methanol: 50 mL

+ Acetic acid: 10 mL + S-1 solution: 1 mL

「Stain」:

Purified water: 100 mL + S-2 solution: 1 mL

「Coloring solution」:

Purified water: 200 mL + S-3 solution: 1 mL

+ S-4 solution: 1 mL

「Stop solution」:

Purified water: 100 mL + Acetic acid: 1 mL

Or Purified water: 100 mL + Citric acid: 3 g

「Cleaning liquid for DTT」:

30 % methanol solution: 100 mL x 2

This solution is necessary if DTT (dithiothreitol) is used for sample preparation.

## 9. Usage

- Method described in the below is for staining a mini slab gel (90 mm x 80 mm, 1 mm thick).

① Put 100 mL fixative in a tray. Immerse polyacrylamide gel after electrophoresis in it and shake for 10 min.

※Prepare 50% bigger tray than gel size.

※Shake the tray strongly enough not to attach gel

and the bottom of tray.

※The processing time of this step is varied by thickness of gel.

- 0.75 mm thick: 5 min
- 1 mm thick: 10 min
- 2 mm thick: 20 min

②Discard fixative and then add 100 mL purified water. After that, shake it for 10 min to wash up the gel.

※If DTT is used for sample preparation, use cleaning liquid for DTT: 100 mL of 30% methanol solution.

※Gel tends to float in cleaning liquid, so that shake it strongly at first then weaken the shaking speed after gel sinks.

※The processing time of this step is also varied by thickness of gel.

- 0.75 mm thick: 5 min
- 1 mm thick: 10 min
- 2 mm thick: 20 min

③Wash up the gel by the same method as Step②.

④With or without DTT, add 100 mL purified water and then shake it for 10 min to wash up the gel.

⑤Discard purified water, add the whole amount of stain and then shake it for 5 min.

※The processing time of this step is varied by thickness of gel.

- 0.75 mm thick: 3 min
- 1 mm thick: 5 min
- 2 mm thick: 10min

⑥Discard stain. Add 100 mL purified water and shake it for 30 seconds to wash up the gel.

※Collect stain as waste liquid including heavy metal.

⑦Discard purified water. Add 100 mL coloring solution and shake it for just 30 seconds.

⑧Discard coloring solution and add rest 100 mL of it again. Shake it a little bit before appropriate stained image appears.

※Time for staining is varied by sample and condition.

※Some color development continues even after stop solution is added in next Step⑨.

※The processing time of this step is varied by thickness of gel. Approximate times are as

following.

- 0.75 mm thick: 5 min
- 1 mm thick: 10 min
- 2 mm thick: 20 min

⑨Add the whole amount of stop solution soon after discarding coloring solution and shake it for 10 min.

⑩Discard stop solution. Add 100 mL purified water and shake it for 5 min to wash up.

⑪Wash up again by the same method as Step⑩.

	Step	Solution	Volume	Time
①	Fixing	Fixative		10 min
②~④	Cleaning	Cleaning liquid		10 min x 3
⑤	Staining	Stain		5 min
⑥	Cleaning	Purified water		30 sec
⑦	Previous coloring	Coloring solution	100 mL	30 sec
⑧	Coloring	Coloring solution		Depending on condition
⑨	Stopping	Stop solution		10 min
⑩~⑪	Cleaning	Purified water		5 min x 2

## 1 0. Supplementary item

- The described each processing time is optimized. Keep the time definitely for operation. If processing time is much longer or shorter than described, it may not color, sensitivity may be reduced and so on.
- When you immerse gel in each solution, confirm the gel is sank completely and then take a step.
- Silver stain is non-specific and 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 it takes 2 days for staining, immerse gel at the 1st day, and then start with washing with purified water at the 2nd day.
- Silver staining can be performed after CBB staining. In the case of that, CBB dye needs to be decolorated completely before silver staining.



**ATTO CORPORATION**  
 3-2-2 Motoasakusa,  
 Taito-ku, Tokyo 111-0041, JAPAN  
 Tel +81 3 5827 4863  
 Fax +81 3 5827 6647  
 E-mail: eig@atto.co.jp  
 http://www.atto.co.jp/eng

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