EzWay™ Protein-Silver Staining Kit

1. Catalog No. K14040D

2. Quantity Tests for 20 mini-gels

3. Storage & Stability Store at R.T.

4. Description The EzWay™ Protein-Silver Staining Kit is based on the chemical reduction of silver ions to metallic silver on a protein band. This kit provides a rapid and easy method for staining proteins in polyacrylamide gels. Silver staining allows detection of most proteins and it is 30-fold more sensitive then staining with Coomassie G-250. Also, this kit specially designed to provide sensitive staining compatible with mass spectrometry analysis.

Quick overview of the silver staining method:

Step 1: Fixing	Removes interfering ions and detergent from the gel and helps to restrict the movements of proteins out of the gel matrix.
Step 2: Sensitizing	Sensitizer increases sensitivity and contrast of the stain
Step 3: Washing	Removes excess Sensitizer and rehydrates the gel using ultra pure water for subsequent staining.
Step 4: Staining	A bind silver ions to the protein and forms a latent image by stainer
Step 5: Washing	Remove excess stainer by ultra pure water
Step 6: Developing	Reduces silver ions to metallic silver at the protein bands resulting in development of the protein bands.
Step 7: Stop	Complexes with any free silver to prevent further reduction.

5. Specification

- Sensitivity: 0.3 ng BSA
- Background must be yellow light and free from dark spots.
- No uneven staining or contaminant bands.

6. Kit Contents

Components	Size
Sensitizer	50 ml
Stainer A	5 ml
Stainer B	5 ml
Developer A	50 ml
Developer B	5 ml

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7. Basic Staining Method

A. Buffer Preparation

Materials Supplied by the user:

- Ultra pure water
- 100 % ethanol
- 100 % Acetic acid

Buffer preparations:

1. **Fixing solution:** Ethanol 40 ml, acetic acid 10 ml and Ultra pure water up to 100 ml. It is recommended to prepare 1 L stock solution.
2. **Second fixing solution:** Ethanol 50 ml and Ultra pure water up to 100 ml. It is recommended to prepare 1 L stock solution.
3. **Sensitizing solution:** Sensitizer 2.5 ml and Ultra pure water up to 25 ml.
4. **Staining solution:** Stainer A 0.25 ml, Stainer B 0.25 ml and Ultra pure water up to 25 ml.
5. **Developing solution:** Developer A 2.5ml, Developer B 0.25 ml and Ultra pure water up to 25 ml.

Note: You may prepare solution before starting or prepare them as you proceed to the next step.

B. Silver Staining Procedure

1. After electrophoresis, remove the gel from the cassette and place it in a clean staining tray of the appropriate size. Rinse the gel briefly with ultra pure water several times.

2. Fix the gel in 50 ml of fixing solution for 15 minutes x 2 times for 1 mm thick gel with gentle rotation.
   Note: The gel can be stored in the fixative overnight if there is not enough time to complete the staining protocol. Longer fixing times may improve the sensitivity and background staining in some cases.

3. Discard the fixing solution and wash the gel in Second Fixing solution for 5 minutes x 2 times

4. Discard the Second Fixing solution and add 25 ml of Sensitizing solution. Incubate the gel in the Sensitizing solution for 2 minutes.

5. Discard the Sensitizing solution and wash the gel in 50 ml of Second Fixing solution for 2 minutes.

6. Wash the gel with 100 ml ultra pure water for 1 minutes x 3 times.

7. Discard the ultra pure water and prepare 25 ml Staining solution. Incubate the gel in the Staining solution for 20 minutes.

8. Discard the Staining solution and wash the gel with 100 ml of ultra pure water for 1 minute x 2 times.

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9. Discard the ultra pure water. Incubate the gel in 25 ml of Developing solution for 1-10 minutes until bands start to appear and the desired band intensity is reached.
   Note: Over develop will cause to strong dark yellow background.

10. Once the appropriate staining intensity is achieved, immediately add 0.5 ml of 100% acetic acid directly to the gel still immersed in Developing solution. Gently, agitate the gel for 10 minutes.

11. Discard the stopped solution and wash the gel with 100 ml of ultra pure water for 30 minutes.

Note: If you having problems with staining or obtaining a light background, please see Section 12. Troubleshooting guide.

8. Trouble Shooting Guide

Problem	Cause	Comments and Suggestion
Dark or uneven background	Poor water quality	Use ultra pure water of >18 MΩ/cm resistance
	Staining trays not clean or containing solutions left over from prior silver staining	Use staining trays dedicated for silver staining. After silver staining, wash trays with soap and water, and rinse them with ultra pure water.
	Improper washing done between steps	Do not skip or reduce any washing steps. If necessary increase immersing time. Alternatively, grater amount.
	Gels are bent or torn	Remove the gels carefully from the cassette after electrophoresis making sure that the gels do not tear. Be careful during handling of the gel.
	Gels are not completely submerged during staining	Be sure to completely immerse gels in staining solution and perform all steps using a rotary shaker for even staining
Poor band development or low sensitivity	Loss of silver ions from the gel	Limit the wash after staining to exactly 1 minute.
	Stainer or developer solution not prepared properly	Make sure that the solutions are prepared correctly using ultra pure water.
	Low protein load	Increase the amount of protein load. Be sure to have at least of 1 ng protein on the gel.
	Short immersing time in the step of sensitizing	Increase immersing time in sensitizing solution.
Fogging appearing during stopping the destaining	Destaining solution was not remover completely	Remove destaining solution completely by using ultra pure water washing before stopping the destaining with 10% acetic acid.

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Stained gels are too dark	Stopper not added to the gel at the appropriate time	Be sure to add the stopper slightly before desired stain intensity is reached.
	Protein is overload	Decrease protein load on the gel
Large dark spots or fingerprints on the gel	Improper gel handling	Always wear gloves while handling gels. Do not apply pressure on the gels while handling.
Presence of a 50-68 kDa band across the gel	Keratin contamination	Wear gloves all times during electrophoresis and staining steps. Rinse all wells of the gel with ultra pure water before sample loading.
Longer time for band developmen t resulting in dark background	Low protein load	Increase the amount of protein load. Be sure to have at least 1 ng protein on the gel.
	Some proteins may need longer fixing time	Increase the time for fixing the gel to 2 hours or overnight.
Negative staining	Protein band is overloaded	Decrease load per band

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