RAPID SILVER STAINING KIT

HIGHLY SENSITIVE KIT FOR FAST STAINING OF PROTEINS & NUCLIC ACIDS

Rapid Silver Staining Kit (Part# REG-RSS-1) from Axygen is designed to stain proteins and polypeptides in polyacrylamide gels with minimal background. The staining kit is 100 times more sensitive then conventional Coomassie stain and requires approximately 10-15 minutes for fixed mini-gels and 60 minutes for regular size gels.

- The protocol is optimized for 0.75 mm to 1.5 mm thick 10-14% polyacrylamide mini-gel at room temperature.
- For regular size 16cm gels, double the time for each step and use twice the volume of each solution.
- For higher % acrylamide mini-gel such as 16-20%, double the time of fixing and reducing steps.
- Staining takes place at gel surface. To enhance surface-to-volume ratio for protein bands, use only thin gels.
- Acetic acid interferes with silver staining, therefore be sure that it is completely washed out of the gel.
- Coomassie stained gels can be re-stained with Axygen's Rapid Silver Stain kit after complete de-staining.
- Clean containers and powder-free gloves should be used while handling the gel.
- Wipe thoroughly the staining tray with the alcohol on a paper towel.
- Use reagent grade double distilled water (conductivity <1 mho) for washes and preparing all solutions.
- It is recommended that all steps are carried out on a gentle shaker or rocker.
- For consistent reproducible results from gel to gel, use constant temperature conditions.
- The staining can be easily performed more efficiently at 37° C in an air-incubator.
- Fixed gel (prior to staining) or stained gels may be stored in re-hydrating solution for several days.
- For long-term storage of the stained gel, drying is recommended.
- Take pictures or photographs of the gel as soon as possible to avoid intensity change.
- Take proper precautions for handling & disposing of Reagent B & D (contains silver nitrate & formaldehyde).
- All proteins do not have a linear response to silver staining.
- Use Axygen special surface staining tray with lid (Part#: SDT 1), which is provided with the kit.

Contents of the Kit

Sufficient for staining 24 mini-gels or 12 regular size gels:

Reagent A	Reducing Solution (2 ml)
Reagent B	Silver Solution (25 ml)
Reagent C	Developer Solution (250 ml)
Reagent D	Developer Enhancer (2 ml)
Special Staining Tray	One Staining Tray with lid for mini-gels

Additional Materials Required:

Fixing Solutions:	50% methanol + 10% acetic acid. Mix 500ml methanol and 100ml glacial acetic acid and add water to make 1 liter.
Rehydration Solution	5% methanol + 7% acetic acid.
Acetic Acid:	Mix 50ml methanol and 70ml glacial acetic acid and add water to make 1 liter.

Procedure:

Fixing Step:

1. Pour approximately 50 ml Fixing Solution in the Staining Tray.
2. Remove the gel from the electrophoresis apparatus and immerse into the Fixing Solution.
3. Cover the tray and gently mix on a shaker for 10 minutes.
4. Decant the fixing solution and add approximately 50 ml re-hydration Solution.
5. Leave on shaker for 5 minutes.

Note: Avoid touching the gel with fingers. Use a spatula to remove the gel from the glass plate. Gel can be stored in the re-hydration Solution for overnight.

Reducing Step:

1. Rinse the gel thoroughly with water 2-3 times (2 to 3 minutes each wash).
2. Add 50 ml of water and one drop of Reagent A (Reducing Solution) directly in the Staining Tray.
3. Leave the gel on shaker for 10 minutes.
4. Acetic acid interferes with the silver staining therefore washing the gel thoroughly is important.

Note: Acetic acid interferes with silver staining, thorough wash is critical.

Staining Step:

1. Decant the Reducing Solution and quickly rinse the gel with water.
2. Add 50 ml water and 1 ml Reagent B (Silver Solution) to the Staining Tray.
3. Leave on the shaker for 10 minutes.

Note: Do not wash the gel for too long after Silver Solution (see next step).

Developing Step:

1. Prepare 50 ml Developing Solution by mixing 10 ml of Reagent C (Developer Solution) with 40 ml water in a small beaker or flask and add one drop of Reagent D (Developer Enhancer).
2. Decant the Silver Solution and quickly rinse the gel with water (Quick rinsing in less than a minute).

3. Add approximately 25 ml of the Developing Solution, mix for 15–30 seconds.
4. Decant completely, add remaining 25 ml of Developing Solution.
5. Mix gently until bands are clearly visible (1–5 minute depending on the amount of the protein).
6. Immediately add 1 ml glacial acetic acid to the Developer Solution and mix to stop the reaction.
7. The staining procedure is now complete.

Note: For heat activated staining, staining steps can be performed at 60°C in microwave for 1minute following fixing of gels.

- The stained gel can be stored in re-hydration solution.
- Silver stain occurs at the surface of the gel. Do not over wash the gel before developing.