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Silver Stain kit

Cat No. SSK20, 20 reactions

Product description:

Silver Stain Kit is an extremely sensitive colorimetric staining procedure for the detection of nanogram amounts of proteins resolved on polyacrylamide gels and efficient destaining of excised gel pieces for mass spectrometry analysis. With high sensitivity and low background, Silver stain kit is ideal for the visualization of protein bands in dilute samples or for the detection of proteins present in trace amounts.

Kit Components:

The solutions included in the Silver Stain Kit are listed below. Sufficient reagents are supplied to stain 1 mini-gels.

Item	Storage	Amount
Solution 1	RT-avoid light	20 ml
Solution 2	Add 20 ml ddH_2O to dissolve. Store at 4°C and avoid light.	4 g
Solution 3	RT	200 ml
Solution 4	Add 400 ul ddH_2O to each tube. Store at 4°C and avoid light.	935 ul
silver destain reagent A	RT-avoid light	2.8 ml
silver destain reagent B	RT	2.8 ml
silver destain reagent C	RT	20 ml
silver destain reagent D	RT	20 ml

Procedure for protein staining in SDS-PAGE gels

Important notes:

- Perform all steps in clean staining tray (plastic or glass) with constant gentle shaking.
- Prepare all solution within 3 min of use.
- Below procedure is for one mini gel use only.

A. Additional materials required for one reaction:

- Fixing solution: 40% ethanol, 10% acetic acid.
- Ethanol wash: 30% ethanol.
- Stop solution: 1.5% EDTA.

B. Procedure

1. **Fixing**- Gel was fixed in 100 ml of 40% ethanol, 10% acetic acid for 40 min (or more to overnight)
2. **Enhancing**- Decant the fixing solution, gel was enhanced in 100 ml of enhance working solution***** for 2 min.

   ***Prepare enhance working solution:** Add 1 ml of **Solution 1** to 99 ml 30 % ethanol and mix thoroughly.
3. **Ethanol wash**- Decant the working solution 1, using 100 ml of 30% ethanol for 2 min.
4. **Water wash**- Decant the 30% ethanol, using 100 ml of ultrapure water for 2 min.
5. Repeat step 4 once.
6. **Silver staining**- Decant the water, gel was stained in stain working solution***** for 4 min.

   ***Prepare stain working solution:** Add 1 ml of **Solution 2** and 150 µl of **Solution 4** to 99 ml of ultrapure water and mix thoroughly.
7. **Quickly wash-** Decant the stain working solution, using 100 ml of water wash for < 20 sec.
8. Repeat step 7 once.
9. **Developer**- Decant the water, immediately develop the gel with the developer working solution*****. Protein bands begin to appear within 30 sec to 3 min. After 3 min, lane background signal may increase to undesirable levels.

   ***Prepare developer working solution:** Add 10 ml of **Solution 3** and 30 µl of **Solution 4** to 90 ml of ultrapure water and mix thoroughly.
10. **Stop**- When the desired band intensity is reached, replace developer working solution with 100 ml of 1.5% EDTA for 10 min.

Procedure for destaining SDS-PAGE gel pieces

Important notes:

- Perform all steps in clean tube with constant gentle shaking.
- Below procedure is for one piece gel or spot (1*1*1 mm) use only.

A. Procedure

1. Excise the stained spot of interest form the gel.
2. Wash gel in 1ml of ultrapure water for 3min.
3. Decant the water, add 70 µl of **Solution A** and 70 µl of **Solution B** to the tube. Gently vortex it for 10 min. Repeat this step once.
   (Do not premix solution A and solution B prior to addition to the tube.)
4. Decant above solution, add 1 ml ultrapure water to the gel and wash for 20 min.
5. Decant the water, add 1 ml **Solution C** to the gel and wash for 5 min.
6. Decant the Solution C, add 1 ml **Solution D** to the gel and wash for 5 min.
7. The gel slice is now ready for further proteomic analysis.

For Research Using Only.
Please do not hesitate to contact us if you have any questions.

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