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Silver Staining for Mass Spectrometry

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1. Wash gel in ultrapure water for 5 minutes twice.

2. Discard water and add Fixing Solution to the gel and incubate for 15 minutes at room temperature twice.

3. Wash gel with the 10% Ethanol Wash for 5 minutes twice.

4. Wash gel in ultrapure water for 5 minutes twice.

5. Just before use, prepare sensitizer working (mix 25µL Sensitizer with 12.5mL water).

6. Incubate gel in sensitizer working solution for exactly 1 minute, then wash twice with ultrapure water for 1 minute each.

7. Prepare silver stain enhancer (mix 0.125mL of enhancer with 12.5mL stain) and immediately add it to the gel then incubate gel for 5 minutes.

8. Prepare developer working solution (mix 0.125mL of enhancer with 12.5mL developer).

9. Quickly wash gel with ultrapure for 20 seconds.

10. Immediately add developer working solution and incubate until protein bands appear (2-3 minutes).

Note: Protein bands begin to appear within 30 seconds and continue to develop. Protein detection vs. background is optimal from 2 to 3 minutes. After 3 minutes, lane background signal may increase to undesirable levels.

11. When the desired band intensity is reached, replace developer working solution with Stop Solution.

Wash gel briefly, then replace acetic acid and incubate for 10 minutes.

12. Immediately proceed to excising and destaining the gel pieces procedure.

De-staining

1. Wash gel in ultrapure water for 10 minutes twice.

2. While using a light box to illuminate the gel, excise protein band with a clean scalpel or spot picker.

3. From a blank region of the gel, excise another gel piece of the same size to use as a control sample.

4. Place gel pieces in clean 0.5mL microcentrifuge tubes.

5. Prepare destain solution by combining 37µL of Silver Destain Reagent A, 122.5µL of Silver Destain Reagent B and 2mL of ultrapure water, which is sufficient to treat 10 gel pieces per lane.

Use this solution within the same day; do not store for prolonged periods.

6. Add 100uL of the destain solution to the gel pieces, mix gently and incubate at room temperature for 15 minutes.

7. Remove the destain solution. Incubate gel pieces in 100ul of additional destain solution for 15 minutes.

If we need to continue working the next day incubate the samples in fixing solution.

8. If we will continue working on the same day remove the destain solution and wash gel pieces three times for 15 minutes each with 0.2mL of Wash Solution.

9. Proceed with in-gel trypsin digestion or other protein elution steps in preparation for the desired mass spectrometry method.

Alternatively, store the gel pieces overnight at -20°C. Do not exceed overnight storage.