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Silver Stain Gel for IP-TDMS Development

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ABSTRACT

Silver stain gels can be used to examine total protein in a given sample. This method is less sensitive than western blotting, but typically more sensitive than Coomassie blue staining.

PROTOCOL CITATION

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MATERIALS TEXT

Sample loading buffer with DTT
Microcentrifuge tube
Hot plate
Centrifuge
Gel box with power source
1 x MES running buffer
Invitrogen 4-12% BisTris gel (10, 12, or 17-well)
Protein MW Ladder
Clean gel box
MilliQ H_2O
Silver Nitrate Fixing Buffer (50% MeOH 45% H_2O, 5% acetic acid)
Silver Nitrate Washing Buffer (50% MeOH, 50% H_2O)
Silver Nitrate Sensitizing Buffer (0.2 g sodium thiosulfate/1 L)
Silver Nitrate Stain (0.05g $AgNO_3$ /50mL H_2O)
Silver Nitrate Developing Buffer (40 g Na_2CO_3, 800 µL formaldehyde/2 L)
Silver Nitrate Terminating Buffer (95% H_2O, 5% acetic acid)

1 Take aliquots from IP fractions and add sample loading buffer to a final concentration of 1X. Boil samples in hot plate at 95ºC for 5-10 min. Briefly, centrifuge samples to ensure sample entire sample is towards bottom of the tube.

2 Open gel and place into gel box. Add 1 x MES running buffer, ensuring that there are no bubbles within the wells of the gel. Carefully load samples, without loss of sample from the well. Make sure that the gel wells have a large enough capacity to contain the entire sample volume. Load at least one lane with 2-3 µL of protein MW ladder. Run the gel at 120-150 V for approximately 40 minutes, or until the sample dye has reached the bottom of the gel.

3 Remove the gel from the cassette and gently wash with deionized water. Cut off the bottom of the gel (where there is a ridge). Place get into clean gel box. Wash gel in MilliQ (MQ) H_2O 3 times for 5 minutes each.

4 Cover with Fixing Buffer for ≥ 20 minutes.

5 Wash in wash buffer for 10 minutes.

6 Wash in MQH_2O for 10 min.

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7 Treat with sensitizing buffer for 1 minute.

8 Wash twice in MQH2O for 1 minute each.

9 Stain $AgNO_3$ for 20 minutes; after this step, all waste must be collected in hazardous waste container.

10 Wash twice in MQH_2O for 20 seconds each.

11 Expose for 1 minute in developing buffer; watch the gel avoid overdeveloping.

12 Treat with terminating buffer for 1 minute; make sure the gel is no longer being developed.

13 Image the gel.

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