Silver staining of SDS-page

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Abstract

Silver staining of a acrylamide gel.

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Guidelines

Fixate-solution:

50 ml. ethanol

12 ml. acetic acid

50 μl filtered 37% formaldehyde

-> 100 ml. H₂O

Thiosulfate-Stock:

20 mg/ml Sodium Thiosulfate

Pre-treatment solution:

1 ml Thiosulfate-Stock:

-> 100ml H₂O

Silver nitrate solution

0.1 g silver nitrate

37.5µl filtered 37% formaldehyde

-> 50ml H₂O

Developer solution:

100µl filtered 37% formaldehyde

40µl Thiosulfate-Stock:

-> 200ml H₂O

12g. Sodium carbonate

Stop-solution

12ml acetic acid

-> 100ml H₂O

Protocol

Fixate the gel.

Step 1.

1 hour (or ON) in 50-100 ml. fixer solution

Wash

Step 2.

Wash the gel

1x20min. in 50% ethanol

2x20min in 30% ethanol

Incubate

Step 3.

Incubate the gel for 1 minute in 100 ml pre-treatment solution

Wash

Step 4.

Wash the gel 3x2sec. with dH₂O

Incubate

Step 5.

Incubate the gel for 20 min in silver nitrate solution

Wash

Step 6.

Wash the gel 3x2sec. with dH₂O

develop

Step 7.

Develop the gel with 100ml developer-solution, replace the developer-solution after 30 sec.

Beware the reaction is very fast!.

Stop

Step 8.

When the protein bands have reached the desired intensity stop the reaction with 100ml of stopsolution

(leave for 10 min)

Step 9.

Incubate the gel in 30% ethanol + 5% glycerol for 30 min. before the gel is dryed