Silver Development protocol (silver stain) for 0.75mm polyacrylamide gels

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Abstract

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Guidelines

What to look for in a silver stain protocol

Avoid silver-ammine stains. Ammonia is touchy stuff and procedures are lengthy. Glutaraldehyde is unnecessary, unless you've got basic proteins (luteovirus capsids? - no data). Be sure gel is thin (0.75mm). Stain should incorporate a sensitizer (dithionite in my case) and a silver chelator (thiosulfate). The latter prevents silver carbonate precipitation. Use high quality distilled water in the last stages of development. Otherwise technical grade chemicals (alcohols, glycine, etc) work fine. I've not successfully stained tricine gels - even with extensive washing; I presume tricine is a better silver precipitant than glycine.

References:

Morrissey, J.H. (1981). Silver stain for proteins in polyacrylamide gels: a modified procedure with enhanced uniform sensitivity. *Analytical Biochem* 17 , 307-31.

Rabilloud, Thierry (2000) Detecting proteins separated by 2-D gel electrophoresis. *Analytical Chemistry* 72 , 48a-55a.

Problem

The protocol is robust. Occasional slow development appears to correlate with old formaldehyde. Doubling the formaldehyde concentration in the developer solves the problem.

Protocol

Step 1.
Agitate gel twice with 50% MeOH, 10% acetic acid (Fixer 1) for 15 min ea.

DURATION
00:15:00

NOTES
Irina Agarkova 24 Mar 2016
Gels can be held for several days in Fixer 1.

Step 2.
Rinse (1 min) with 200 ml DW.

DURATION
00:01:00

Step 3.
Agitate in 10% EtOH, 5% glacial acetic acid (Fixer 2) for 6 min.

DURATION
00:06:00

NOTES
Irina Agarkova 24 Mar 2016
We use commercial (cheap) alcohols.

Step 4.
Agitate in distilled water. Agitate twice (9 min each) with 500 ml DW.

DURATION
00:09:00

NOTES
Irina Agarkova 14 Apr 2016
Thorough rinsing gives a low, uniform background.

Step 5.
Agitate in 500 ml of 20 mg/L $Na_2S_2O_4$ (hydrosulfite [dithionite], make fresh) in distilled, deionized water (DDW) for 9min (sensitization).

NOTES
Irina Agarkova 24 Mar 2016
Caution - the half-life of dithionite solution is tens of minutes.

Step 6.
Pour off solution.

Step 7.
Without rinsing, add 200 ml, 0.1% $AgNO_3$ (200 mg) in DDW.

Step 8.
Add 150 ml 37% formaldehyde.

Step 9.
Agitate for 9 min.

DURATION
00:09:00

Step 10.
Rinse 30 sec with DDW to remove excess $AgNO_3$.

DURATION
00:00:30

Step 11.
Add 200 ml image developer (1ml 37% formaldehyde per liter of 3% sodium carbonate) mixed with 200 ml 10g/L sodium thiosulfate.

Step 12.
Agitate to desired staining intensity (3 to 6 min).

DURATION
00:06:00

NOTES
Irina Agarkova 24 Mar 2016
Thiosulfate complexes silver preventing silver carbonate precipitation.

Step 13.
Pour off developer.

Step 14.
Add 80 ml of stop solution (50 g tris, 25 ml glacial acetic per liter of distilled water).

Step 15.
Store gel in 10% glycerol with bacteriostat (0.02% NaN_3).

Warnings

If you're thinking of using thicker gels - don't.

Caution: Avoid thiols; they release peptides from keratin creating ' rain '. For protein gels reduce and alkylate samples (with 5mM DTT [see advice on DTT], and 15mM iodoacetamide respectively)