

Protocol 103 _ Sensitive silver staining of LPS

Reagents

100% ethanol
Glacial acetic acid
Glacial periodic acid
Concentrated ammonium hydroxide
0.1N sodium hydroxide
Silver nitrate
Citric acid
37% formaldehyde
Glass dish for staining

Protocol:

NB: Avoid any extraneous contamination of the gel – very clean gel plate, handle with ethanol-rinsed gloves, very clean staining vessel and glass-ware for making the stain.

Fix LPS in polyacrylamide gel by placing into 40% EtOH-5% acetic acid in a clean glass dish overnight

Replace the fixing solution with 0.7% periodic acid in 40% EtOH-5% acetic acid to oxidize for 5 mins

Wash 3 times, each for 15mins with 500ml-1000ml water

Prepare staining reagent:

2ml conc ammonium hydroxide added to 28ml of 0.1N NaOH.
5ml of 20% silver nitrate is added whilst the solution is being stirred (transient brown precipitate will form)
115ml water is added to make 150ml staining reagent

Drain thoroughly and pour in staining reagent. Agitate vigorously for 10mins

Wash 3x for 10mins in 500-1000ml water

Prepare developer:

1l water plus 50mg citric acid and 0.5ml of 37% formaldehyde
(only 200ml will be required for one gel, remainder can be stored)

Replace water with developer and develop until desired intensity is achieved (2-5mins typically). Stop developing by removing the gel to water and washing twice.