Ag-LPS staining procedure

JOURNAL OF BACTERIOLOGY, Vol. 154, No. 1. Apr. 1983, p. 269-277

1. Overnight fixation in200 ml of 25% (vol/vol) isopropanol in 7% (vol/vol) acetic acid
   25 ml IPA + 70 ml H2O + 5.25 ml Acetic acid

2. 5-min oxidation in 150 ml of distilled water with 1.05 g of periodic acid and 4 ml of 25% (vol/vol) isopropanol in 7% (vol/vol) acetic acid (solution made up just before use).
   25% IPA=7.5ml H2O+2.5 ml IPA
3. Eight 30-min washes, each time with 200 ml of distilled water
4. 10-min silver staining in a solution consisting of 0.1 N NaOH (28 ml), concentrated (29.4%) ammonium hydroxide (1ml), 20% (wt/vol) silver nitrate (5 ml), and distilled water (115 ml) (make up solution just before use and stir constantly while making)

0.2 g NaOH in 50 ml of H2O (0.1N NaOH) , 1 g Silver nitrate

5. four 10-min washes, each time with 200 ml of distilled water
6. 10 to 20 min of developing in 250 ml of developer solution (citric acid [50 mg], 37% formaldehyde [0.5 ml], distilled water [amount sufficient to make 1 liter of solution]; made up just before use) at an optimal temperature of 25°C (if solution cools, staining of protein as well as LPS will occur)

25 mg Sodium sitrate + 0.25 ml formaldehyde + 500 ml H2O

7. 1 h in a stop bath (200 ml of distilled water plus 10 ml of 7% [vol/vol] acetic acid)

200 ml H2O + 0.7 ml AcH

8. Final wash with 200 ml of distilled water and then storage (gel may be stored in a zip-lock plastic bag with a small amount of water to prevent desiccation).

Note : Use of concentrated (29.4%) ammonium hydroxide is essential for the preferential staining of LPS. To maintain the quality of this reagent, we place small amounts of ammonium hydroxide from a freshly opened bottle into small (25- to 50-ml) bottles with caps that can be tightly sealed. Loss of "strength" of the reagent results in persistence of the brown precipitate in the staining solution. Addition of more ammonium hydroxide will dissolve the precipitate; however, preferential staining of LPS will not result.