LPS SILVER STAIN #2

REFRENCES: Tsai and Frasch. J. Bact. 1982
Tsai and Frasch. Anal. Biochem. 119:115 – 119, 1982.

OBJECTIVE: Preferred method for LPS gel staining

METHOD:

1. Soak gel overnight in 40% ethanol (for outer membrane or cell envelopes, substitute isopropanol for ethanol to amplify LPS) and 5% acetic acid in 200 ml of distilled water.

2. Soak the gel for 5 minutes in periodic acid solution to oxidize the LPS.

0.7% periodic acid	1.4g
40% ethanol (or isopropanol)	84.2 ml
5% acetic acid	10.0 ml
Make up to 200 ml with distilled water	

3. Rinse in running distilled water for 2 hours.

4. Put developer in the 37° room to warm up.

5. Add staining reagent and agitate for 10 minutes.

2 ml concentrated ammonium hydroxide (use fresh frozen stock)
28 ml 0.1N NaOH
5 ml 20% silver nitrate (add dropwise to above with vigorous stirring)
115 ml distilled water

6. Rinse and wash 3 times in distilled water (15 min/wash).

7. Change to a new dish and add warm developer.

10 mg citric acid
0.1 ml 30% formaldehyde
200 ml distilled water

8. Stop the staining reaction by reaction by washing with 0.35% acetic acid (1.4 ml glacial acetic acid in 400 mls dH_2O). Fix in this solution for at least one hour. Store in distilled water.

9. Destain as for protein silver stain.