

LPS PAGE with Silver Stain

Materials:

Lysis buffer

Reagent	In 1L
SDS	2g
2-Mercaptoethanol	4mL
Bromophenol Blue	0.003g
1M tris-Cl pH6.8	to 100mL

SDS-PAGE gel

10% separating gel	4% stacking gel
5.3mL 30% acrylamide/0.8% bisacrylamide	650µL 30% acrylamide/0.8% bisacrylamide
6.5mL water	3.05mL water
3.75mL 4x Tris-Cl/SDS pH 8.8	1.25mL 4x Tris-Cl/SDS pH 6.8
2.88g Urea	25µL 10%APS
50µL 10%APS	5µ TEMED
10µL TEMED	

Fixation Solution:

Reagent	for 200mL
isopropyl alcohol	50mL
Acetic Acid	14mL
ddH ₂ O	up to 200mL

Oxidation Solution:

Reagent	for 150mL
Periodic acid	1.05g
EtOh	4mL
Acetic acid	500µL
ddH ₂ O	146mL

Staining Reagent

-on a 50mL Falcon tube

Reagent	
1M NaOH	2mL
Ammonium hydroxide	2.6mL
ddH ₂ O	41mL

- on a 15mL Falcon tube

Reagent	
AgNO ₃	1g
ddH ₂ O	5mL

Developing Solution

Reagent	
1% citric acid	2mL
Formaldehyde	100 μ L
ddH ₂ O	200mL

Stop Solution

Reagent	
Acetic Acid	1.6mL
ddH ₂ O	up to 200mL

Methods:

Preparing the samples:

1. Resuspend cells on PBS.
2. Make a 1 mL 1:100 aliquot of the cells.
3. Determine OD at 600nm.
4. Calculate $(5/OD)1000$ = amount of cells needed for 1mL 5OD resuspension.
5. Spin down at 8krpm for 5 min
6. Resuspend in 200 μ L Lysis buffer by pipetting up and down.
7. Boil for 10 mins.
8. Add 3 μ L 20 μ g/ μ L Proteinase K; incubate at 60C for 1h.

Making the gel:

1. Install equipment.
2. Mix the reagents for the separating gel.
3. Pour separating gel, cover with a layer of ddH₂O; wait for 45 mins or until gel polymerizes. Take out water layer.
4. Mix reagents for stacking gel, and pour. Avoid bubbles.
5. Insert comb. Allow to polymerize for 45 mins or until gel polymerizes.
6. Install gel in the equipment. Add 1x SDS running buffer.
7. Load 6 μ L of Kaleidoskope Marker, and 20 of each sample. Run at 35mAmp for 2h.

LPS Silver stain:

1. Place the gel in fixation solution ON at 4C
2. Replace with oxidation solution for 5 mins with gentle agitation
3. Wash gel in 200 μ L ddH₂O for 30 mins, repeat 3 times. Change to a clean glass dish after rinses.
4. Mix the two components of the staining reagent (pour silver nitrate slnt into the 50mL falcon tube SLOWLY; a brown ppt will for briefly, but dissolves quickly). Remove the last wash and add fresh staining reagent. Shake for 10 minutes)
5. Wash the gel in ddH₂O for 10 mins X4.
6. Add developing soln for 10-20 mins, The LPS bands appear slowly until yellow background staining of gel appears.
7. Stop development by washing on Stop solution.
8. Rinse the gel in ddH₂O, store in the dark at 4C.