

SDS-PAGE

Materials

1. 1.5mL + 15mL tubes
2. Pipets
3. SDS-PAGE gel ingredients
4. Gel box
5. SDS power apparatus
6. Sonicator
7. Tabletop centrifuge
8. Heat block
9. Buffers
10. ...

SDS-PAGE sample preparation

1. Resuspend pellet with 400uL 300mM NaCl and 50mM HEPES 5% glycerol pH 7.4
2. Sonicate each sample 4 times for 10 seconds on lowest setting
3. Centrifuge @ 4°C for 30:00 min.
4. Transfer 50uL supernatant into 1.5mL tube (soluble fraction) + discard rest of s.n.
5. Resuspend pellet with 100uL dH₂O
6. Transfer 50uL supernatant into 1.5 mL tube
7. Add 10uL SDS sample buffer to all 1.5mL tubes
8. Boil @ 98°C for 10:00 min + spin down

SDS Gel Making Protocol

1. Make Separating and Stacking gel mixtures
2. Take 1mL of separating mixture into 1.5mL tube
3. Add 4uL TEMED to 1.5mL tube
4. Add 500uL separating mixture + TEMED in between glass plates
5. Add isopropanol to even out gel line
6. Wait ~ 30 min for gel to solidify
7. Add 8-10uL TEMED into remaining separating solution
8. Add separating + TEMED soln. between glass plates up to 1cm away from top
9. Add isopropanol to even out gel line
10. Wait ~ 30 min for gel to solidify
11. Add 8uL TEMED to stacking mixture and add in between glass plates (overflow it)
12. Add comb and wait ~30 min for gel to solidify
13. Add SDS Running buffer, load wells (10uL) and run gel

SDS-PAGE Gel Running Conditions

14. 80V until proteins are halfway down gel
15. 160V until 2cm away from bottom
16. 200V until blue dye runs off

SDS-PAGE Gel Washing + Staining Protocol

17. Wash gel with dH₂O 3 times and microwave for 90 seconds (45 seconds per gel)

18. Remove dH₂O and add blue dye + stir for 10 min
19. Wash off dye and wash with dH₂O 3 times
20. Add 5 kimwipes (clump into ball) and place in corner of Tupperware
21. Microwave for 3:00 min
22. Stir for 10 min
23. Image

40% Polyacrylamide SDS-PAGE gel recipe

Ingredients	Stacking	Separating
Gel percentage (%)	5	10
40% Polyacrylamide (mL)	0.5	2.5
1M Tris(mL) Stacking: pH 6.8 Separating: pH 8.8	0.5	2.5
10% Ammonium persulfate (mL)	0.04	0.1
10% SDS (mL)	0.04	0.1
TEMED (mL)	0.004*	0.004*
dH ₂ O (mL)	2.92	4.8
Total volume (mL)	4	10

Separating (1 gel) Recipe

- 2mL 30% acrylamide
- 2.95mL PAGE-mix
- 50uL 10% APS
- 2uL TEMED

Stacking (1 gel) Recipe

- 0.325mL 30% acrylamide
- 2.175mL STACK-mix
- 25uL 10% APS
- 2.5uL TEMED