Native Discontinuous PAGE

Reference

1. Afreen, S., & Ferreira, A. (2022). The formation of small aggregates contributes to the neurotoxic effects of tau_{45-230}. Neurochemistry international, 152, 105252. https://doi.org/10.1016/j.neuint.2021.105252

Solutions

1. Tris-HCl: 1.5 M Tris, pH 8.8 using HCl
2. Tris-HCl: 500 mM Trish, pH 6.8 using HCl
3. Tris/Glycerol Sample Buffer: 125 mM Tris, 149 nM Bromophenol Blue, 331 nM Pyronin Y, 20% (v/v) Glycerol, 0.01% (v/v) NP-40
4. Tris/Glycine Electrophoresis Buffer: 192 mM Glycine, 25 mM Tris

Protocol

(Limited to proteins that are negatively charged at neutral pH – should work for 17kDa because of its isoelectric point)

1. Prepare Gels. Each gel should have a different acrylamide concentration so that the size of the standards can be used to estimate the size of your unknown (17 oligomer). The below table shows the amount to make for particular percent acrylamide gels. These make 10 mL for 1 gel (makes 20 mL for our 2-gel pouring system).

Stock Solution	5%	7.5%	10%	12%
Acrylamide	1.67	2.5	3.33	4
4X Tris-HCl pH 8.8	2.5	2.5	2.5	2.5
Water	5.83	5	4.17	3.5
10% APS	33.3 µl	33.3 µl	33.3 µl	33.3 µl
TEMED	6.67 µl	6.67 µl	6.67 µl	6.67 µl
Total	10 ml	10 ml	10 ml	10 ml

2. Stacking Gel: 0.65 mL Acrylamide + 1.25 mL Tris (pH 6.8) + 3.05 mL water + 25 µl 10% APS + 5 µl TEMED.
3. Mix tissue in 300 µl Tris-Glycerol Buffer.
   a. Homogenize on ice
   b. Centrifuge at 16,000 x g for 30 minutes at 4°C
   c. Add DNase to supernatant at final concentration 1-2 Units/µl and $MgCl_2$ at 2mM
      - Add 24 µl of 25 Units/µl DNase to 300 µl
      - Add 1.21 µl 0.5 M $MgCl_2$
   d. Incubate at room temperature for 30 minutes
   e. Centrifuge at 16,000 x g for 30 minutes at 4°C and collect supernatant
5. Prepare native standards according to Sigma's instructions.

6. Connect Power Supply and run at 15 mA. This should take ~2 hours.
7. Transfer gel to membrane in our standard Electrophoresis transfer buffer. Do immunoblotting on portion that has the human lysate and stain the portion with standards with PonceauS (Coomassie blue might be more permanent)
    - Invitrogen protocol suggests that to increase transfer efficiency of native proteins, incubate gel in 0.1% Sodium Dodecyl Sulfate (SDS) for 15 minutes before immunoblotting.
8. Estimate relative mobilities of each protein (migration distance of band/migration distance of dye front). This is taken from the 1-D Electrophoresis paper in folder.
9. Plot log Rf (y) against gel concentration (x). Determine slope (Kr).
10. Plot –log Kr of each line (y) against log of molecular weight of standards (y). Use this line to estimate size of unknown.