Electropurification of Proteins

This protocol is for electrically eluting pure proteins from SDS-page gels. This is useful for unstructured proteins that are highly protease sensitive.

1. Concentrate initially purified protein to ~1-2 mg/mL (by Bradford assay).

2. Obtain Novex 10% TG gels with 2D wells (1 big well + 1 small well for standards). Alternatively, you can use a pipet tip to remove the well dividers from a standard gel.

3. Combine 250uL concentrated protein of interest + 125uL 2x Morris buffer (+BME).

4. Heat 10 minutes at 95˚C, then spin briefly in tabletop mini-centrifuge.

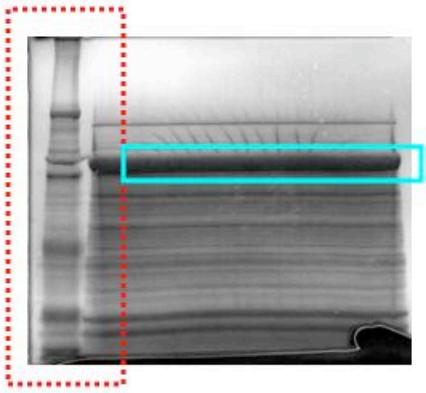
5. Load the entire 375uL into the big well of the gel. Load standards in small well.

6. Run gel as always.

7. While gel runs, prepare Elution Solution:
   1 Litre:
   6.06g Tris Base
   0.08g DTT
   0.37g EDTA (free acid, not Na-salt)
   1.00g SDS
   ddH_2O to 1L
   pH should be ~9.0.
   Filter through 0.22um filter

8. Zinc stain gel
   -Wash gel quickly with ddH_2O
   -Shake 15 minutes in solution A (200mM imidazole, 0.1% SDS)
   -Wash quickly with ddH_2O
   -Shake ~30 seconds in solution B (200mM $ZnSO_4$) until the band appears (the gel turns white where protein is NOT)
   -Wash with ddH_2O to stop staining.
   -If desired, scan the gel in the scanner with a black paper background

Zinc-stained gel of Nsp1



9. Using a scalpel, cut strips out of the gel as indicated in the picture.

   a. The strip indicated by the red dotted line will be stained with commassie blue and used to show what the band looked like originally and where it was in relation to the standards.
   b. The strip indicated by the blue solid line contains the full-length protein of interest. This strip will be used for the purification.

10. Destain the protein band in 1x running buffer (contains SDS). Meanwhile, boil dialysis tubing (Spectra/Por MWCO 6-8,000, 1.7mL/cm, 14.6mm diameter) in 50mM EDTA in the microwave, then wash with ddH_2O.

11. Fill a flat DNA gel box with Elution Solution.

12. Clip the bottom of a length of dialysis tubing (1.5x length of band).

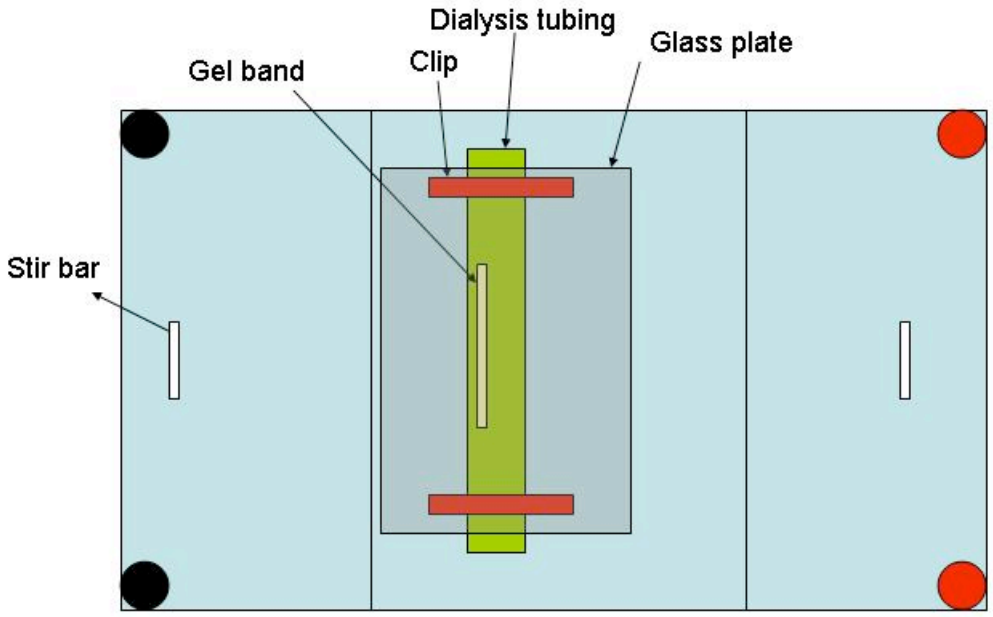
13. Fill the tubing with elution solution, and put the gel band inside.

14. When the gel band sinks to the bottom, carefully pour the buffer out of the tubing, leaving the gel band at the bottom.

15. Add Elution Solution to the tubing for a total of 4-7mL (less is better).

16. Clip closed, so that no air is inside and that it's filled with buffer.

17. Set up the electropurification as in the following picture:



The glass plate sits on top of the dialysis tubing to prevent the dialysis tubing from floating up above the elution solution. Try to get the gel band to be towards the negative end of the tubing.

18. Run in the cold room, overnight, at 80V, 33mA.

19. The next day, the protein should have eluted out of the gel band. Transfer the elution solution from the dialysis tubing to a falcon tube. TCA/DOC precipitate protein or concentrate in centrifuge concentrator. Determine concentration by Brandford assay.

20. Coomassie stain the protein band after electroelution to see how much protein is left in the band.