# Dialysis Protocol

| final conc. | | Buffer I | Buffer II | Stock |
|---|---|---|---|---|
| 6 *M* | Urea | 72g | | |
| 20 m*M* | HEPES pH 7.9 | 4mL | 40mL | 1 *M* |
| 300 m*M* | KCl | 30mL | 44.76g | 2 *M* |
| 0.1 % | NP-40 | 2mL | 20mL | 10 % |
| 5 m*M* | $MgCl_2$ | 1mL | 10mL | 1 *M* |
| 10 % | Glycerol | 20mL | 200mL | 100 % |
| 1 m*M* | DTT | 200µL | 2mL | 1 *M* |
| 0.5 m*M* | PMSF | 1mL | 10mL | 100 m*M* |
| 10 µ*M* | $ZnSO_4$ | 200µL | 2mL | 10 m*M* |
| | fill w/ $H_2O$ | 200mL | 2 L | |

NOTE: add the $ZnSO_4$ if dealing with a Zn-finger protein

Procedure:

1. cut desired length of dialysis tubing; take note of the MW cut-off of the tubing
2. Boil tubing for 2' in $dH_2O$ to sterilize
3. Soak tubing in Buffer I
4. Clamp up one end, open the other end, add some Buffer I into tubing, check for leaks
5. Remove buffer and add the protein sol'n. leave some space at top and clamp up.
6. Mix gently and let spin by stir bar slowly for 2hrs in the cold room (4C).
7. Dialyze as follows:

| Step | Urea | | Time | Total V |
|---|---|---|---|---|
| A | 6M | buffer I | 2 hrs. | 200mL |
| B | 5M | add 35mL buffer II | 2 hrs. | 236mL |
| C | 4M | add 50mL buffer II | 2 hrs. | 286mL |
| D | 3M | add 75mL buffer II | 2 hrs. | 361mL |
| E | 2M | add 120mL buffer II good O/N point | 2 hrs. | 481mL |
| F | 1M | substract 250mL, add 250mL of buffer II | 2 hrs. | 500mL |
| G | 0.5M | substract 250mL, add 250mL of buffer II | 2 hrs. | 500mL |
| H | 0M | dump all out, add buffer II only | 2 hrs. | 500mL |
| I | 0M | dump all out, add buffer II only | 2 hrs. | 500mL |

8. Transfer tubing to buffer III and spin for 2hrs. @ 4C (cold room)

| Buffer III: | | | | |
|---|---|---|---|---|
| | 20mM | HEPES pH7.9 | 20mL | 1M |
| | 100mM | NaCl | 20mL | 5M |
| | 0.1% | NP-40 | 10mL | 10% |
| | 5mM | $MgCl_2$ | 5mL | 1M |
| | 10% | Glycerol | 100mL | 100% |
| | 1mM | DTT | 1mL | 1M |
| | 0.5mM | PMSF | 5mL | 100mM |
| | 10µ*M* | ZnSO4 | 1mL | 10m*M* |
| | fill w/ $H_2O$ to | | 1L | |

9. Open tubing and transfer contents to 15mL conical vial
10. Freeze on dry ice or liquid nitrogen and store at –80C to concentrate later.