Protein dialysis protocol

A. Note：

Before entering the laboratory, put on facemasks and gloves.

B. Theory：

1. Semipermeable membrane is a kind of membrane that allows certain molecules to pass through. In dialysis, we utilize this property to select certain proteins in the dialysis bag.
2. There are a lot of coefficients that affects the efficiency of dialysis:
   - pH
   - Temperature
   - Time
   - Buffer solution volume
   - Thickness of dialysis membrane

C. Materials：

- NaCl, Tris-HCl, Glycerol, ddH_2O
- Dialysis bag
- A few Clips

D. Laboratory procedures：

1. Dialysis buffer formula
   - Prepare 3L of dialysis buffer (Dialysis can be divided into 3 stages, and each stage would need 1L of dialysis buffer.)
   - Target concentration: NaCl-150 mM, Tris-HCl-20mM, glycerol 20%
   - Store at 4℃
2. Dialysis
   - After cutting the proper length of the dialysis bag, soak in dialysis buffer
   - Clip the bottom of the dialysis bag with metal clip to seal well. Add the protein into the dialysis bag, and get rid of all bubbles. Clip the other end with plastic clip and tie an eppendorf onto it.

     Note: The dialysis bag should be suspended in the dialysis buffer, thus the two side of the dialysis bag would be clipped with different kind of clips.
   - Put a stir bar in the dialysis buffer and stir at 4℃

     Note: Stir bar would attract the metal clip by magnetic force; the dialysis bag would rotate with the stir bar.
   - Change dialysis buffer every 3 hours. The last stage of dialysis can be stored overnight.
   - Collect samples from dialysis bag
   - Centrifuge 13000 rpm, 4℃, 20 mins
   - After centrifuging, obtain the supernatant and store at -20℃