Making the PAGE gels

1. Use alcohol and Kimwipes to wipe the glass, and then set up the rest of the apparatus
2. For small proteins, use a higher percentage gel
3. Prepare the resolution gel solution without ammonium persulfate or TEMED.
   Note: If the weather is cold in the room, you may wash the solution with hot/warm tap water to help the polymerization
4. Invert tube gently after adding ammonium persulfate and TEMED, then pour the solution to the mark.
5. Overlay the monomer solution with t-butanol. Allow the gel to polymerize 45-60 min.
6. Pour off t-butanol and rinse the top of the gel with distilled water then dry the area.
7. Place the comb and pour the stacking gel solution until all the teeth are filled up by the solution.
8. Allow the gel to polymerize 30-45 min.

Preparing and Loading Samples

1. Add an equal volume of 2X SDS sample buffer to protein sample.
   Note: Keep on ice to avoid proteins getting destroyed.
2. Heat the sample to 100℃ for 5 minutes.
3. Centrifuge at 14,000 rpm in a microcentrifuge for 5 min.
4. Load the appropriate volume of your sample onto the SDS-PAGE gel.

Running and transferring the gel

1. Run the gel at 100V through the stacking part of the gel and increase the voltage up to 200V after the proteins have gone through the stack.
2. Allow migration to continue until the blue dye front is at the end of the glass plates, but has not migrated off the gel to finish the run.
3. Wet the PVDF membrane with methanol for 30 seconds, place it in distilled water, and then filled with transferring buffer as well as fiber pads and filter papers.
4. Cut off stacking gel and soak it in transferring buffer.
5. Assemble the transfer sandwich and the blot should be on the cathode and the gel on the anode.
   Note: Remove air bubbles by rolling a glass tube on the membrane to avoid any air bubbles are trapped in the transferring sandwich.

6. Put the cassette in the transfer tank, insert the ice cooling and fill with transferring buffer in the chamber.
7. Transfer at 4℃ for 1-2 hours.
8. Stain with Pongee S to check the transfer quality. (This step is optional.)

Blotting

1. Block the blot in 5% non-fat milk in PBST at room temperature for 1 hour or overnight at 4℃.
2. Wash the blot 3-5 times for 5 minutes with PBST.
3. Incubate the blot with diluted primary antibody at room temperature for 1-3 hours or overnight at 4℃.

   Note: Conditions depends on antibody quality and performance.
4. Wash the blot 3-5 times for 5 minutes with PBST.
5. Incubate the blot with diluted secondary antibody at room temperature for 1 hour.
6. Wash the blot 3-5 times for 5 minutes with PBST.