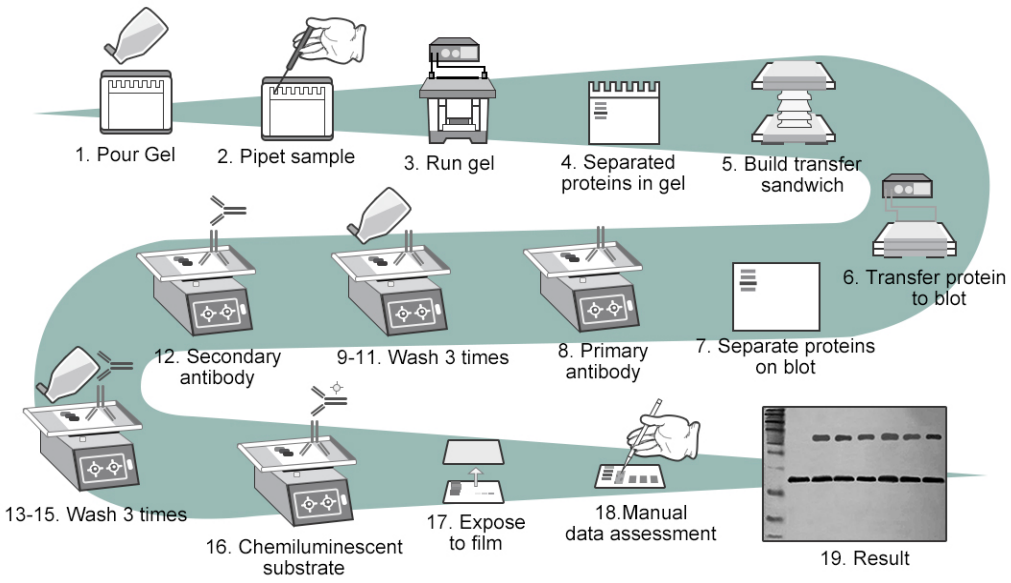
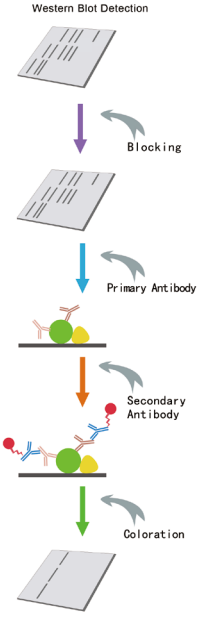
Principles for Western Blot

Western Blotting is used to identify the large molecular antigen (usually protein) that can interact with specific antibodies and determine the MV of the antigen. The protein was separated by SDS electrophoresis gel, and then transferred to solid phase support by electrophoresis. Solid phase support includes nitrocellulose membrane, poly vinylidene fluoride two (PVDF) membranes and cationic nylon membrane. The unreacted sites on the membrane were sealed up to inhibit the nonspecific adsorption, so that the immobilized proteins could interact with specific primary antibodies and then the secondary antibodies which conjugated with enzyme or fluorescein. Finally, it is located by means of radiation, chromophore or chemiluminescence.





Western Blot reagents

1. 1.0 mol/L Tris • HCl （pH6.8）
2. 1.5 mol/L Tris • HCl（pH8.8）
3. 10% SDS
4. 10% Ammonium persulfate（APS）
5. 30% Acrylamide
6. Reduced 5×SDS (Loading Buffer)
7. Electrophoretic Buffer
8. Transfer Buffer
9. TBS Buffer
10. Eluant TBST Solution
11. Blocking Buffer（TBST buffer with 5% skimmed milk or 5% BSA）
12. Antibody dilution buffer: Usually using blocking buffer as antibody dilution buffer which contain 5% skimmed milk or 5% BSA, Each antibody has its best dilution ratio.

Steps of the experiment

1. Prepare the gel (separation gel) according to the molecular weight of protein.
2. Using appropriate lysates and methods to prepare samples. Adding samples.
3. Electrophoresis： Usually in inconsecutive system, the voltage of stacking gel (80V is suggested) is lower than the voltage of separation gel (110-150V is suggested)
4. After Electrophoresis, cut off the useless gel which has no sample, the sponge mat and filter paper soaked by transferring buffer.
5. Transfer the proteins to membrane:
   Choose appropriate membrane: take PVDF membrane as example
   Prepare PVDF membrane, soak in Methanol for 5s
   Put black plate - fiber mats - filter paper - gel -PVDF membrane - filter paper - fiber mat - white plate in order successively.
6. Choose the constant current, the electric current of each tank is suggested to be 150-200mA. Adjust the time according to molecular weight of the protein.
7. Blocking: Take out the rinsed membrane, put it into the blocking buffer (5% skim milk powder or 5% BSA). Incubate on the shaker at room temperature for 2 hours.
8. Incubation of antibody
   Add diluted primary antibody to blocked membrane, incubate over night at 4℃ .
9. Wash the membrane with washing buffer for 10 minutes and repeat 3 times.
10. Add the diluted secondary antibody, incubate at room temperature for 2 hours.
11. Wash the membrane for 10 minutes and repeat 3 times.
12. ECL preparation: mix the substrate A and B as the ratio 1:1. Prepare when needed.
13. Cover the blotting membrane with mixed substrate for 1-5 minutes, observe the fluorescence in dark.
14. Record the result with machine or in dark room with autoradiography film or chemiluminiscence imaging system.
15. For autoradiography film, expose for several seconds to minutes according to the fluorescence intensity. The exposed film will be soaked in developing liquid till strips appear, then rinse in the fixing solution. Wash the film with water and hang up to dry
16. Analyze the bands with Bandscan software