SDS-PAGE

(Poly Acrylamide Gel Electrophoresis)

Reagents:
Laemmli Buffer

1) Clean a set of glass plates (the one is flat, the other has spacer) with 100% Ethanol. Use Kimwipe to clean plates. Dry them up for few seconds.

2) Set glass plates as described in the Bio-Rad direction. The side you clean is inside.

3) Prepare the Lower (Separating) Gel solution (See recipe). Pour the gel solution between the glass plates. Cover the gel surface with 0.25ml of 2-propanol. Wait for 20 to 30 min until the gel becomes solidified.

4) Discard the 2-propanol layer. Wash the gel surface with MilliQ water, twice. Remove all the remaining water with a piece of 3MM paper. But don't touch the surface of Lower Gel.

5) Prepare Upper (Stacking) Gel solution (see recipe). Pour with the upper gel solution on top of the solidified Lower gel with a comb in between the plates. Wait for 20 to 30 min until the gel becomes solidified.

6) Set the gel sandwich into Bio-Rad Mini-PROTEAN 3 Cell system. Fill the chamber with Laemmli Buffer. Don't mix inner buffer and outer buffer.

7) Prepare your protein samples. Mix well and centrifuge to collect the liquid at the bottom of the tube. Thaw the Pre-Stained Protein Ladder Marker. Mix well and centrifuge to collect the liquid at the bottom of the tube.

8) Apply marker and samples with Hamilton Syringe (15ul for a large gel, 8ul for a mini gel). The applying volume of your samples are depend on a case.

9) Put the lid on. Start running the gel.

35 mA constant current for 1 gel for about 30 min
50 mA constant current for 2 gel (one container) for about 30 to 40 min
or
You can reduce current to run the gel slowly. For example:
25 mA constant current for 1 hour

10) Run the gel until the front line reaches at the bottom.