Laemmli SDS PAGE

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[Abstract] Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is used to separate proteins with relative molecular mass no smaller than 10 KD. Very small proteins (<10 KD) are difficult to resolve due to low ability of binding to SDS, which can be solved by gradient gels or using different eletrophoresis conditions, like Tricine-SDS-page. The basic Laemmli SDS PAGE procedure is described here.

Materials and Reagents

1. Pre-stain Protein MW marker (Bio-Rad Laboratories)
2. TEMED (Life Technologies, Gibco®)
3. Ammonium persulfate (Sigma-Aldrich)
4. SDS (Research Organics INC)
5. 30% Acrylamide stock (37.5: 1 acrylamide: bisacrylamide) (Bio-Rad Laboratories)
6. Bromophenol Blue (Thermo Fisher Scientific)
7. Tris Base (Calbiochem-Behring)
8. Glycine (EM Science)
9. EDTA (Amresco)
10. Glycerol (EM Science)
11. Isopropanol
12. Tris-HCl (pH 6.8)
13. β-mercaptoethanol (Sigma-Aldrich)
14. 10x running buffer (see Recipes)
15. 2x SDS protein sample buffer (see Recipes)

Equipment

1. Protein mini gel cassettes (Bio-Rad)
2. Heating block module
3. Table-top centrifuge
4. Power supply
5. Gloves

6. Filter paper

Procedure

A. Making SDS-PAGE gel

1. Clean and completely dry glass plates, combs, and spacers are required.
2. Assemble gel cassette by following manufacturer instructions.
3. Prepare 10% lower gel (separating gel) by adding the following solutions (wear gloves when prepare gel solution) (total volume= 5 ml)
   2 ml ddH_2O
   1.67 ml 30% acrylamide/Bis
   1.25 ml 1.5 M Tris (pH 8.8)
   25 µl 20% SDS
   25 µl 10% ammonium persulfate (make it fresh and store at 4 °C up to a month)
   2.5 µl TEMED (add it right before pour the gel)
   Note: Change ration of ddH_2O to 30% acrylamide/Bis to get different percentage of separating gel.
4. To avoid polymerization, after adding TEMED, mix well and quickly transfer the gel solution by using 1 ml pipette to the casting chamber between the glass plates and fill up to about 0.7 cm below the bottom of comb when the comb is in place.
5. Add a small layer of isopropanol to the top of the gel prior to polymerization to straighten the level of the gel.
6. Once the gel has polymerized, start to prepare stacking gel (5%) by adding the following solutions (total volume= 3 ml)
   2.088 ml dH_2O
   0.506 ml 30% acrylamide/Bis
   0.375 ml 1 M Tris (pH 6.8)
   15 µl 20% (w/v) SDS
   15 µl 10% ammonium persulfate
   1.5 µl TEMED (add it right before the gel is poured)
7. Remove the isopropanol layer by using filter paper. Rinse the top layer of the gel with ddH_2O and dry off as much of the water as possible by using filter paper.
8. Add TEMED and mix the stacking gel solution well. Quickly transfer the gel solution by using a 1 ml pipette till the space is full, and then insert the appropriate comb.
9. Allow the top portion to solidify and then carefully remove the comb.

Note: The gels can be stored with the combs in place tightly wrapped in plastic wrap and put in a second container with wet tissue towel (keep the gels moist) at 4 °C for 1 to 2 weeks.

B. Sample preparation

1. Prepare same amount of protein samples according to BCA assay result, see BCA (bicinchoninic acid) protein assay.
2. Add the same volume of 2x protein sample buffer to each protein sample, mix and boil the samples at 95 °C heating block module for 10 min.
3. Spin the samples at the maximal speed for 1 min (samples from some tissue/cell sources may need longer spin) in tabletop centrifuge and leave the samples at room temperature until you are ready to load onto the gel.
   Note: Can store extracted protein samples (containing sample buffer) at -20 °C and reheat at 95 °C for 5 min when used the following time.

C. Electrophoresis

1. Remove the gel cassette from the casting stand and place it in the electrode assembly with the short plate on the inside. Press down on the electrode assembly while clamping the frame to secure the electrode assembly and put the clamping frame into the electrophoresis tank.
2. Pour some 1x electrophoresis running buffer into the opening of the casting frame between the gel cassettes. Add enough buffer to fill the wells of the gel. Fill the region outside of the frame with 1x running buffer.
3. Slowly load the same amount of protein samples into each well as well as load 10 µl of protein MW marker.
4. Connect the electrophoresis tank to the power supply.

D. Protein detection

If protein of interest is about 0.2 µg or more in the sample, typically use Coomassie blue staining (see Coomassie blue staining). Otherwise, use silver staining (sliver staining), which is more sensitive and can detect as little as 5 ng protein.

Recipes

1. 10x running buffer
   30.3 g Tris-base
   144.0 g glycine

10.0 g SDS

Completely dissolve in about 800 ml ddH_2O and then more ddH_2O up to 1 liter.

2. 2x SDS protein sample buffer

   1.25 ml 1 M Tris-HCl (pH 6.8)

   4.0 ml 10% (w/v) SDS

   2.0 ml glycerol

   0.5 ml 0.5 M EDTA

   4 mg bromophenol blue

   0.2 ml b-mercaptoethanol (14.3 M)

   Bring the volume to 10 ml with ddH_2O.

References

1. Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227(5259): 680-685.

