Protocol 2-2: Protein Identification SDS-PAGE

General Protocol

1) Pouring the separation gel
    a) Assemble gel sandwich according to the manufacturer's instructions, or according to the usage of alternative systems. For Mini-Gel, be sure that the bottom of both gel plates and spacers are perfectly flush against a flat surface before tightening clamp assembly. A slight misalignment will result in a leak.
    b) Combine solutions C and B and water in a small Erlenmeyer flask or test tube.
    c) Add ammonium persulfate and TEMED, and mix by swirling or inverting container gently (excessive aeration will interfere with polymerization). Work rapidly at this point because polymerization will be under way.
    d) Carefully introduce solution into gel sandwich using a pipet. Pipet solution so that it descends along a spacer. This minimizes the possibility of air bubbles becoming trapped with the gel.
    e) When the appropriate amount of separating gel solution has been added (in the case of the Mini-Gel, about 1.5cm from top of front plate or 0.5cm below level where teeth of comb will reach), gently layer about 1cm of water on top of the separating gel solution. This keeps the gel surface flat.
    f) Allow gel to polymerize (30-60 min). When the gel has polymerized, a distinct interface will appear between the separating gel and the water, and the gel mold can be tilted to verify polymerization.
2) Pouring the stacking gel
    a) Pour off water covering the separating gel. The small droplets remaining will not disturb the stacking gel.
    b) 2) Combine Solution C and B and water in a small Erlenmeyer flask or a test tube.
    c) 3) Add ammonium persulfate and TEMED and mix by gently swirling or inverting the container.
    d) 4) Pipet stacking gel solution onto separating gel until solution reaches top of front plate.
    e) 5) Carefully insert comb into gel sandwich until bottom of teeth reach top of front plate. Be sure no bubbles are trapped on ends of teeth. Tilting the comb at a slight angle is helpful for insertion without trapping air bubbles.
    f) 6) Allow stacking gel to polymerize (about 30 min).
    g) 7) After stacking gel has polymerized, remove comb carefully (making sure not to tear the well ears).
    h) 8) Place gel into electrophoresis chamber.
    i) 9) Add electrophoresis buffer to inner and outer reservoir, making sure that both top and bottom of gel are immersed in buffer.
3) Protein sample preparation

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a) Protein sample is diluted with an equal volume of sample buffer. In the experiment, take 10ul samples and 10ul sample buffer. The mixture is heated for 5 min at 100℃. Introduce sample solution into well using a Hamilton syringe. Layer protein solution on bottom of well and raise syringe tip as dye level rises. Be careful to avoid introducing air bubbles as this may allow some of sample to be carried to adjacent well. Rinse syringe thoroughly with electrode buffer or water before loading different samples. Include molecular weight standards in one or both outside wells. A slab gel is especially useful for molecular weight determinations since the sample and molecular weight standard proteins can be run under identical conditions on a single gel. There are a number of commercially available SDS-PAGE molecular weight standards which give a good spread of molecular weight lines in a gel.

4) Running a gel

a) Attach electrode plugs to proper electrodes. Current should flow towards the anode.

b) Turn on power supply to 200V.

c) The dye front should migrate to 1cm from the bottom of the gel in 30-40 min for two 0.75mm gels (40-50 min for 1.5mm gels).

d) The high electrical current used in gel electrophoresis is very dangerous. Never disconnect electrodes before first turning off the power source. If using an electrophoresis apparatus, which is not completely shielded from the environment, always leave a clearly visible sign warning that electrophoresis is in progress.

e) Turn off power supply.

f) Remove electrode plugs from electrodes.

g) Remove gel plates from electrode assembly.

h) Carefully remove a spacer, and inserting the spacer in one corner between the plates, gently pry apart the gel plates. The gel will stick to one of the plates.

5) Stain and destain the gel

a) Stain the gel in staining solution for overnight at RT.

b) Destain the gel, change fresh destaining solution several times.

Reference

6) Sambrook J, Maniatis T, Fritsch EF. Molecular Cloning: a Laboratory Manual. cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 3rd ed., 2001.

7) Robert F. Weaver. Molecular Biology, McGrawHill, 4th edition, 2007

8) http://2009.igem.org/Team:Tsinghua/Protocol

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