Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is a method to resolve proteins in a mixture based on their molecular size. Negatively charged sodium dodecyl sulfate (SDS) in the sample buffer binds to heat denatured proteins. The proteins migrate toward the positive pole in an electrical gradient, impeded by the polymerized and cross-linked polyacrylamide. This protocol calls for Novex precast gels.

**Procedures**

1. Place the mini-gel buffer core into the lower buffer chamber.
2. Place the gel tension wedge into the lower buffer chamber.
3. Remove the gel cassette from the gel pouch.
4. Rinse the gel cassette with deionized water.
5. Peel the tape off of the bottom of the cassette.
6. Pull the comb out of the cassette with care.
7. Place one or two gels in the Mini-Cell in such a way that the notched well side of the cassette faces the buffer core (if only one gel is being run, the molded buffer dam replaces the second gel cassette).
8. Clamp the gels in the lower buffer chamber by pulling the gel tension wedge forward.
9. Fill the upper buffer chamber with 1X running buffer (approximately 200 ml), ensuring that all the wells are full of running buffer and that air bubbles are displaced from the wells.
10. Check for leakage of the 1X running buffer from the upper to the lower buffer chamber. In case of leakage, repeat steps 7 through 9.
11. Load (underlay) the samples into the wells with 20-µl pipette tips. Note: use of special narrow pipette tips can maximize the volume that can be loaded by starting at bottom of the well and slowly raising tip during loading process. Load unused wells with sample buffer used to prepare the samples. Be sure to load each lane with the same volume to prevent the appearance of uneven width of protein bands. For blots that will be processed with phosphospecific antibodies, load at least one lane with a positive control sample (examples given under Reagents and Materials).
12. Fill the lower buffer chamber with 1X running buffer (approximately 600 ml).
13. With the power OFF, place the lid on the buffer core. The lid can be firmly placed only if the negative electrode is aligned correctly with the banana plug on the right.
14. Connect the electrode cables to the power supply.
15. Turn on the power supply and run the gels, usually held at 130 volts for 90 min.
16. When the run is complete (dye front near bottom of gel), shut off the power supply, disconnect the electrodes, and remove the gels from the Mini-Cell.
Reagents and Materials

Novex Precast Tris-HCl Gels: Invitrogen; catalog number varies based on the concentration of acylamide used to produce gel. Concentration is chosen based on the sizes of proteins of interest.

XCell SureLock Mini-Cell: Invitrogen; catalog no. EI0001
   Includes lid, gel tension wedge, lower buffer chamber, mini-gel buffer core, and molded buffer dam
SDS-PAGE running buffer, 1X (1X running buffer), 1 L: AfCS Solution Protocol PS00000055

Example positive control samples for blots processed with phosphospecific antibodies:
Preparation of B Cell Positive Control A for Western Blot Analysis, AfCS Solution Protocol PS00000083 or Preparation of B Cell Positive Control B for Western Blot Analysis, AfCS Solution Protocol PS00000085

Author: Erica Turon

Date: 04/24/02

Approved: Susanne Mumby