

SDS-PAGE—Bio-Rad Criterion Cell
AfCS Procedure Protocol PP0000000300
Version 1, 02/6/02

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 Bio-Rad precast gels.

Procedures

1. Remove Criterion precast Tris-HCl gel cassette from storage container.
2. Rinse gel cassette with deionized water.
3. Peel the tape off of the bottom of the cassette.
4. Pull the comb out of the cassette with care.
5. Place one or two gels into the slots in the tank of the Criterion Cell, ensuring that the upper buffer chamber of the gel is facing toward the center of the cell.
6. Fill the upper buffer chamber with 1X running buffer (approximately 60 ml), making sure that all the wells are full of running buffer and that air bubbles are displaced from the wells.
7. Load (underlay) the samples into the wells with 20- μ l 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 the same SDS-containing 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.
8. Fill the lower buffer chamber with 1X running buffer (approximately 800 ml) up to the "fill" line marked on the side of the tank.
9. Place the lid on the tank after ensuring that the power is off. Make sure to align the color-coded banana plugs and jacks according to color.
10. Connect the electrode cables to the power supply.
11. Turn on the power supply and run the gels, usually held at 130 volts for 90 min.
12. Shut off the power supply, disconnect electrodes, and remove gel cassettes from the tank when the run is complete (dye front near bottom of gel).

Reagents and Materials

Criterion precast Tris-HCl gels: Bio-Rad; catalog number varies based on the concentration of the acylamide used to produce gel. Concentration is chosen based on the sizes of proteins of interest.

Bio-Rad Criterion Cell: Bio-Rad; catalog no. 165-6001
Includes tank, lid with power cables, and sample loading guides

SDS-PAGE running buffer 1X (1X Running buffer), 1 L: AfCS Solution Protocol ID
PS0000005500

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Date: 02/15/02

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