

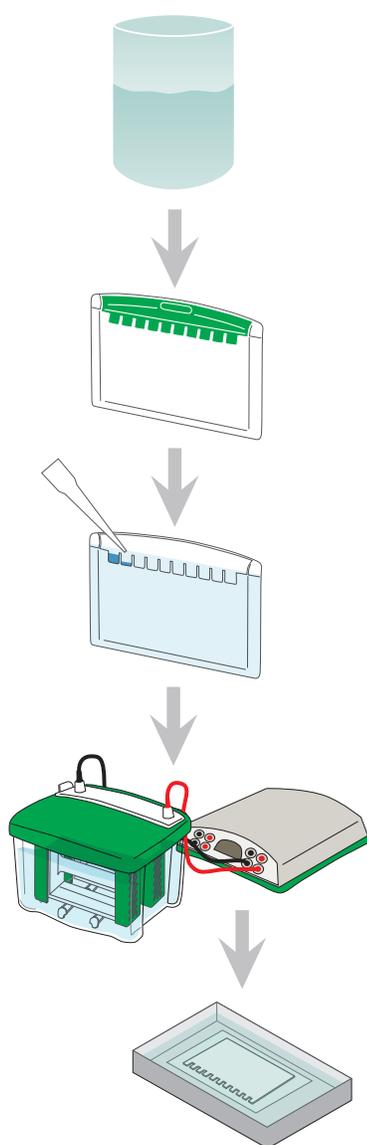
Performing Electrophoresis

Protocol

Bulletin 6202

General Protocol: SDS-PAGE

The following is a generalized protocol for running a Mini-PROTEAN® TGX™ gel in the Mini-PROTEAN Tetra cell. For detailed instructions, refer to the Mini-PROTEAN precast gels instruction manual and application guide (bulletin #1658100).



- 1 Prepare buffers:
 - a. Running buffer (1×): Dilute 100 ml of 10× stock with 900 ml diH₂O.
 - b. Sample buffer: Use Laemmli sample buffer.

- 2 Prepare gels and assemble the electrophoresis cell:
 - a. Remove the comb and tape from the gels and assemble the electrophoresis cell.
 - b. Fill the inner and outer buffer chambers with running buffer. Fill the upper (inner) buffer chamber of each core with 200 ml of 1× running buffer. Fill the lower (outer) buffer chamber to the indicator mark for 2 gels (550 ml) or 4 gels (800 ml) with 1× of running buffer. At runs >200 V, fill the outer buffer chamber to the 4-gel (800 ml) mark.

- 3 Prepare samples as indicated in the table below.

Component	Reducing	Nonreducing
Sample	5 µl	5 µl
Laemmli sample buffer	4.75 µl	5 µl
β-mercaptoethanol	0.25 µl	—
Total volume	10 µl	10 µl

- 4 Heat samples at 90–100°C for 5 min (or at 70°C for 10 min). Load the appropriate volume of your protein sample on the gel.

- 5 Connect the electrophoresis cell to the power supply and perform electrophoresis according to the following conditions:

Run conditions: 200 V
Run time: 31–39 min
Expected current (per gel): Initial 35–50 mA
Final 20–31 mA

- 6 After electrophoresis is complete, turn the power supply off and disconnect the electrical leads. Pop open the gel cassettes and remove the gel by floating it off the plate into water.

- 7 Stain and image the gel, using one of the protocols on the following pages as examples.

BIO-RAD

This is an excerpt from Bio-Rad's comprehensive Electrophoresis Guide (Bulletin 6640).



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