SDS-PAGE PROTOCOL

Adapted from Current Protocols, Ch. 10

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Materials

To Pour Gels:

- 30% acrylamide
- 10% SDS
- 10% APS (make fresh each time)
- TEMED
- 1.5 M Tris, pH 8.8 (resolving gel)
- 1.0 M Tris, pH 6.8 (stacking gel)

5x SDS Running Buffer (1 L)

Tris	15 g
Glycine	72 g
SDS	5 g

Coomassie Blue Stain

- 10% (v/v) acetic acid
- 0.006% (w/v) Coomassie Blue dye
- 90% ddH_2O

Isopropanol Fixing Solution

- 10% (v/v) acetic acid
- 25% (v/v) isopropanol
- 65% ddH_2O

SDS sample loading buffer (40 ml)

ddH_2O	16 ml
0.5 M Tris, pH 6.8	5 ml
50% Glycerol	8 ml
10% SDS	8 ml
2-βmercaptoethanol	2 ml (add immediately before use)
bromophenol blue	

10% (v/v) acetic acid

Protocol

1. Prepare polyacrylamide gel according to standard protocol.
2. Load samples and run gel @ 25 mA (2 gels run @ 50 mA) in 1x SDS Running Buffer.
3. At this point, the gel can either be transferred to a membrane (see Western protocol) or stained with Coomassie (see below).
4. Place gel in a plastic container. Cover with isopropanol fixing solution and shake at room temperature. For 0.75 mm-thick gels, shake 10 to 15 min; for 1.5 mm-thick gels, shake 30 to 60 min.
5. Pour off fixing solution. Cover with Coomassie blue staining solution and shake at RT for 2 hr.
6. Pour off staining solution. Wash gel with 10% acetic acid to destain, shaking at RT ON.

WESTERN BLOT

Adapted from protocol accompanying Hybond ECL Membrane

Materials

Transfer Buffer

1x SDS Running Buffer in 20% Methanol

1x PBS/0.1% Tween 20

Blotting buffer, store at 4 ºC

5% milk in 1x PBS/0.1% Tween 20

Protocol

1. Run SDS-PAGE.
2. Wet membrane in H2O. Soak membrane in transfer buffer for 10 min.
3. Set up transfer from the gel to a nylon membrane in transfer buffer.
4. Place "transfer sandwich" in semi-dry transfer chamber. Run at 23 V for 30 min for 0.75 and 1.0 mm gels or 40 min for 1.5 mm gel.
5. Block blot by soaking in blotting buffer for 1 hr with shaking. **Alternatively**, blocking can be done with as much as 10% milk and 0.5% Tween 20 to reduce background.
6. To 10 ml blocking solution, add primary antibody at proper dilution. Incubate the membrane for 1 hr with shaking. **Alternatively**, incubation with 1º Ab can be done ON @ 4 ºC,
7. Wash 2x briefly with PBS-T, then wash 3x with PBS-T for 5 min.
8. To 10 ml PBS-T, add secondary antibody at proper dilution. Incubate the membrane for 1 hr with shaking.
9. Wash 2x briefly with PBS-T, then wash 3x with PBS-T for 5 min.
10. Detection by ECL. Expose blot to film for 15 sec – 5 min.