

## Total Protein Detection

General protocols are described below. For more details, refer to the instruction manual for the stain you are using.

### SYPRO Ruby Stain

Consult the SYPRO Ruby Protein Stains Instruction Manual (bulletin 4006173) for complete instructions. Membranes stained with SYPRO Ruby protein blot stain are best preserved by allowing membranes to air dry.

- 1 Pretreat** — after electroblotting proteins to a nitrocellulose membrane, completely immerse the membrane in 7% acetic acid and 10% methanol, and incubate for 15 min.
- 2 Wash** — wash the membrane in diH<sub>2</sub>O four times for 5 min each.
- 3 Stain** — completely immerse the membrane in SYPRO Ruby protein blot stain for 15 min.
- 4 Wash** — wash the membrane in diH<sub>2</sub>O four to six times for 1 min each. Monitor the membrane periodically using UV epi-illumination to determine the level of background fluorescence.
- 5 Image** — image using epi UV or green excitation.
- 6** Proceed to immunodetection (if needed).

### Ponceau S Stain

- 1 Stain** — incubate the membrane for 1–2 min in the staining solution.
- 2 Destain** — destain the membrane in water until the background clears. Ponceau S staining is reversible and will disappear after extended destains in diH<sub>2</sub>O.
- 3 Wash** — rinse the membrane in TBS or deionized H<sub>2</sub>O before drying.
- 4** Proceed with immunodetection.

### Colloidal Gold Total Protein Stain

Consult the Bio-Rad enhanced colloidal gold total protein detection kit manual for complete instructions.

- 1 Wash** — following transfer or protein application, wash the membrane three times for 20 min in high-Tween TBS (TTBS with 0.3% Tween 20).
- 2 Water wash** — wash the membrane for 2 min in deionized H<sub>2</sub>O to remove interfering buffer salts.
- 3 Stain** — incubate the membrane with colloidal gold stain, completely covering the blot. Incubation times will vary with the concentration of protein present on the membrane. Most bands will be visible in 1–2 hr. If increased sensitivity is required, continue the assay using the gold enhancement procedure.

This is an excerpt from Bio-Rad's comprehensive Protein Blotting Guide (Bulletin 2895).



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