3. Transfer the membrane to an incubation vessel and wash for 20 minutes with 100 ml of the TTBS solution. Discard the solution and repeat the wash two more times.
4. Add 100 ml of DI water to the incubation vessel. Rinse the membrane for 2 minutes. Discard the water and repeat the water rinse step two more times.

   Note: The water rinse is critical to remove all salts that might interfere with the colloidal gold staining.
5. Add enough colloidal gold total protein stain to the vessel to completely cover the membrane (approximately 50 ml or 0.2 ml per cm^2). Incubation times will vary with the concentration of protein present on the membrane. Concentrated protein bands will begin to appear in minutes, and all bands should be visible in 1–2 hours. Overnight incubation might increase assay sensitivity, but there is also a possibility that background staining will increase.
6. When staining is satisfactory, remove the colloidal gold solution from the membrane. Rinse the membrane for 1 minute in 100 ml of DI water. Decant the water and repeat the rinse step two more times.

   Note: The colloidal gold solution is a reusable reagent. After staining, store the used portion in a separate, clean plastic container in the refrigerator. The colloidal gold total protein stain can be reused until the gold is depleted, as evidenced by the loss of the dark burgundy color and longer staining times.

Section 4
Ordering Information

Catalog Number	Product Description
170-6527	**Colloidal Gold Total Protein Stain,** 500 ml
161-0715	**Tris,** 100 g
161-0716	**Tris,** 500 g
170-6531	**Tween 20,** 100 ml
170-6435	**Tris-Buffered Saline,** 10x liquid concentrate, 1 L

Bio-Rad Laboratories, 2000 Alfred Nobel Dr., Hercules, CA 94547
LIT294 Rev D

Colloidal Gold Total Protein Stain

Catalog Number
170-6527

BIO-RAD

Bio-Rad's colloidal gold total protein stain is a stabilized colloidal gold solution, optimized for rapid and sensitive identification of proteins bound to nitrocellulose or PVDF membranes.[1] Protein bands stain dark red following incubation of the membrane with the colloidal gold solution. The stained membrane yields a permanent record of the protein pattern for exact comparison to immunostained results. The colloidal gold total protein stain is provided ready for use; no reconstitution or dilution is required.

This reagent is recommended for staining proteins bound to Bio-Rad's nitrocellulose or PVDF membrane. To detect proteins bound to a positively charged nylon membrane, use the Biotin-Blot™ Protein detection kit (catalog number 170-6512).

1. Rohringer, R. and Holden, D.W., *Anal. Biochem.,* **144,** 118–127 (1985).

Section 1 Specifications

Contents	500 ml
Storage	4 °C; do not freeze.
Shelf life	6 months at 4 °C

Section 2 Materials and Reagents Required but Not iIncluded

- Incubation vessel–shallow container large enough to lay membrane flat
- Orbital or rocking shaker
- Deionized water
- 10x TBS buffer (Bio-Rad)
- Tween 20
- NaCl—Reagent grade (optional)
- HCl—Reagent grade (optional)
- Tris (optional)

Section 3 Assay Procedure

The following procedure is sufficient to assay one 15 x 15 cm nitrocellulose (PVDF) membrane with 50 ml of the colloidal gold total protein stain. The volume of colloidal gold solution should be adjusted to match the size of the incubation vessel and specific membrane being stained. (It is recommended to use the colloidal gold total protein stain at a volume of approximately 0.2 ml per cm^2 of membrane.)

Perform all wash and incubation steps at room temperature on a moving shaker platform. Make sure that the membrane is completely immersed in solution during the entire assay.

Note: Deionized water of less than 1 µohm conductivity is recommended for the wash steps. Contaminants such as chloride ions will cause non-specific precipitation of the colloidal gold solution.

1. Prepare a Tween-Tris buffered Saline (TTBS) solution. (20 mM Tris 500 M NaCl, 0.3% Tween 20, pH 7.5) Use either premixed buffer or prepare solution from reagent grade chemicals:

   10x premixed buffer (catalog number 170-6435) Add 200 mL of 10x TBS to 1794 mL of DI water. Then add 6 ml of Tween 20. Mix thoroughly.

   Reagent grade chemicals:
   Add 4.84 g Tris and 58.44 g NaCl to 1.9 L of distilled, deionized water. Adjust the pH to 7.5 with HCl. Add 6 ml of Tween 20 and adjust the volume to 2 L with dd water.

2. Bind proteins to Bio-Rad's nitrocellulose or PVDF membrane by electrophoretic transfer, dot blotting, or microfiltration methods.