5. Add 50 ml of Colloidal Gold Total Protein Stain to the vessel to completely cover the membrane. 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 incubations might increase assay sensitivity, but there is also a possibility that background staining will increase.
6. When staining is satisfactory, remove the gold sol from the membrane. Rinse the membrane for 1 minute in 100 ml of dd water. Decant the water and repeat the rinse step two more times.

   **Note:** The gold sol 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.
7. At this point the colloidal gold staining procedure is complete. If increased sensitivity is desired, the Gold Enhancement Kit (catalog number 170-6538) should be used. The components of this kit are used to deposit metallic silver on top of the gold particles present on the membrane surface. The silver metal turns the protein bands black, producing sharper contrast and enhanced detection sensitivity. For more information about the entire assay, consult the instruction manual provided with the Enhanced Colloidal Gold Total Protein Detection Kit (catalog number 170-6517).



## Ordering Information

| Catalog Number | Product Description |
|---|---|
| 170-6517 | **Enhanced Colloidal Gold Total Protein Detection Kit**, includes 500 ml Colloidal Gold Total Protein Stain, Tris, 100 g, Tween-20, 100 ml, Gold Enhancement Kit, and instruction manual |
| 170-6527 | **Colloidal Gold Total Protein Stain,** 500 ml |
| 170-6538 | **Gold Enhancement Kit,** includes silver lactate, 5 g, hydroquinone, 50 g, citric acid, anhydrous, 250 g, sodium citrate, dihydrate, 250 g, fixing solution, 16 oz |
| 161-0715 | **Tris,** 100 g |
| 161-0716 | **Tris,** 500 g |
| 170-6531 | **Tween-20,** 100 ml |
| 170-6435 | **Tris-Buffered Saline,** 10x liquid concentrate |

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LIT294 Rev C

# Colloidal Gold Total Protein Stain

## Catalog Number 170-6527

BIO-RAD

Bio-Rad's Colloidal Gold Total Protein Stain is a stabilized gold sol optimized for rapid and sensitive identification of proteins bound to nitrocellulose 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 prior to use. For increased detection sensitivity, the optional Gold Enhancement Kit (catalog number 170-6538) may be used in conjunction with the total protein stain.

This reagent is recommended for staining proteins bound to Bio-Rad's nitrocellulose or PVDF membrane. To detect proteins bound to Immun-Lite™ 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 Assay Procedure

The following procedure is sufficient to assay one 15 x 15 cm nitrocellulose membrane with 50 ml of the Colloidal Gold Total Protein Stain. The volume of gold sol 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 rotating shaker platform. Make sure that the membrane is completely immersed in solution during the entire assay.

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



1. Prepare the following solution:

   Tween-20, Tris-Buffered Saline, 1x TTBS, 2 L:

   (20 mM Tris, 500 mM NaCl, 0.3% Tween-20, pH 7.5)

   Add 4.84 g Tris and 58.44 g NaCl to 1.9 liters 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.

   **Note:** Premixed 10X liquid concentrate Tris-Buffered Saline (catalog number 170-6435) is also available.

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

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 dd water to the incubation vessel. Rinse the membrane for two 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.