4. **First antibody** - decant the TTBS and incubate with first antibody solution (antibody dilution buffer: 1% gelatin in TTBS) for 1 to 2 hours with gentle agitation.
5. **Washes** - decant the first antibody solution and wash the membrane twice in TCBS (0.05% Tween-20 in CBS [20 mM citrate, 500 mM sodium chloride, pH 5.5]) for 5 minutes per wash with gentle agitation.
6. **Protein G gold** - immerse the membrane in the conjugate solution and incubate for sufficient time (4-24 hours) to achieve satisfactory sensitivity.
7. **Washes** - decant the protein G gold solution and return the conjugate solution to its container; the solution may be reused until its performance deteroriates. Wash twice in TCBS for 5 minutes per wash and once with CBS. Rinse the membrane with deionized water.
8. **Optional enhancement** - following the final rinse, the membrane may be dried, or the detection sensitivity may be increased by using Bio-Rad's Gold Enhancement Kit, catalog 170-6538.

Bio-Rad Laboratories, 2000 Alfred Nobel Dr., Hercules, CA 94547
LIT-334 Rev B

Blotting Grade Protein G Gold Conjugate

Catalog 170-6426

BIO-RAD

Specifications

Contents	2.0 ml
Buffer	20 mM citrate, 150 mM NaCl, pH 5.5, containing 0.1% bovine serum albumin, 0.1% Tween-20, and 0.02% azide as a preservative. The solution has an absorbance of 5 at 520 nm.
Method of preparation	Protein G is conjugated to gold particles using a modification of the method described by Brada and Roth.[1]
Storage conditions	**Do not freeze.** Store refrigerated at 4-8 °C.
Shelf life	Six months at 4 °C.
Recommended working dilution	Dilute the conjugate 1:25 in a 20 mM citrate, 150 mM sodium chloride, pH 5.5 buffer, containing 0.1% bovine serum albumin, 0.1% Tween-20, 0.4% gelatin, and 0.02% sodium azide. The solution may be reused until performance deteriorates. Use plastic containers for dilution, incubation, and storage. Glass containers will reduce the shelf life of the product.

Note: When preparing the Protein G gold dilution buffer, insure that the pH of the buffer is greater than 4.0 before adding sodium azide. Toxic hydrazoic acid is formed at a pH less than 4.0.

This product is intended for research use only. It is not intended for clinical diagnostic purposes.

Reference

1. Brada, D. and Roth, J., *Anal. Biochem.*, **142,** 79 (1984).

Abbreviated Immun-Blot® Protein G-Gold Procedure

Protein G gold is used for the detection of membrane bound antigen-antibody complexes. For complete instructions, order the Immun-Blot assay kit, catalog 170-6428. The following steps should be performed at room temperature.

1. **Antigen application** - apply the antigen to the membrane by dot-blotting, electrophoretic transfer, or microfiltration blotting.
2. **Blocking** - immerse the membrane in blocking solution (3% gelatin in TBS [20 mM Tris, 500 mM sodium chloride, pH 7.5]). Incubate for 1 hour with gentle agitation.
3. **Wash** - decant the blocking solution and wash the membrane in TTBS (0.05% Tween-20 in TBS) for 5 minutes with gentle agitation.