gelatin in TCBS]) and incubate 1-2 hours with gentle agitation.

7. Decant the Protein G-HRP solution. Wash the membrane twice for 5 minutes in TTBS with gentle agitation.
8. Rinse the membrane once for 5 minutes in TBS.
9. Prepare the color development solution just prior to use:
   a. Dissolve 60 mg HRP development reagent into 20 ml ice cold methanol. Protect from light.
   b. Add 60 ml ice cold 50% H_2O_2 (hydrogen peroxide) to 100 ml RT TBS. Mix this with (a) above. Use immediately. This will produce a 0.015% H_2O_2-development solution.
10. Develop the membrane in the color development solution for 5-30 minutes with gentle agitation. Remove the membrane to a water wash when a satisfactory signal is generated.

Bio-Rad Laboratories, 2000 Alfred Nobel Dr., Hercules, CA 94547
LIT417 Rev C

Blotting Grade Protein G - Horseradish Peroxidase Conjugate

Catalog Number
170-6425

BIO-RAD

Specifications

Contents	1.0 ml
Buffer	10 mM phosphate, 150 mM NaCl, pH 7.4. Contains 1.0% bovine serum albumin and 0.01% thimerosal.
Preparation of conjugate	A 1:1 mixture of Protein G and horseradish peroxidase was conjugated by a modification of the method of M. Wilson and P. K. Nakane.[1]
Storage	This reagent is shipped frozen on dry ice, and can be stored at -20 °C prior to opening. Once thawed, store the reagent at 4 °C. Repeated freeze-thaw cycles will damage the reagent.
Shelf life	One year at 4 °C.
Recommended working dilution	1:3,000

This product is intended for research use only. It is not intended for clinical diagnostic purposes. No CAS available.

Reference

1. Wilson, M. B. and Nakane, P. K., Immunofluorescence and Related Staining Techniques, Knapp, W., Holubar, K. and Wicks, G., eds., Elsevier/North Holland Biomedical Press, Amsterdam, pp. 215-244 (1978).

Abbreviated Immun-Blot® Protein G-HRP Procedure

For complete instructions, order the Immun-Blot® protein G-HRP assay kit or call 1-800-4BIORAD. Reading the entire instruction manual is advised for optimum results and avoidance of most common problems.

Note: All steps are performed at RT.

1. Prepare the nitrocellulose blot, *i.e.* electrophoretic blotting, passive dot-blotting, or filter lifts.
2. Block the membrane in blocking solution (3% gelatin in 20 mM Tris, 500 mM NaCl, pH 7.5 [TBS]) for 1 hour with gentle agitation.
3. Decant the blocking solution and wash the membrane in TTBS (0.05% Tween-20 in TBS) 5 minutes with gentle agitation.
4. Decant the wash and incubate with diluted first antibody solution with gentle agitation (antibody dilution buffer: 1% gelatin in TTBS).
5. Decant the first antibody solution. Wash the membrane twice for 5 minutes in TCBS (0.05% Tween-20 in 20 mM citrate, 500 mM NaCl, pH 5.5) with gentle agitation.
6. Add the diluted protein G-HRP solution (1:3,000 in Protein G dilution buffer [1%