

- 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<sub>2</sub>O<sub>2</sub> (hydrogen peroxide) to 100 ml RT TBS. Mix this with (a) above. Use immediately. This will produce a 0.015% H<sub>2</sub>O<sub>2</sub>-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.



## Blotting Grade Protein G - Horseradish Per- oxidase 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. <sup>1</sup>
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%