

3. Immerse the membrane in the DAB color development solution. Protein concentrations of at least 100 ng will become visible immediately as brown bands or dots. Lesser concentrations may take as long as 15 minutes. Do not overdevelop, as high backgrounds may result.
4. Stop the color development by immersing the membrane in dd H₂O for 10 minutes. Change the water at least once to remove remaining color development solution.

Reagent Storage	Temperature	Shelf Life
HRP Color Development Reagent, DAB	-20 °C	1 year
TBS solution	RT	1 month



Instructions for Preparation of
DAB (3,3'-diaminobenzidine)
Color Development Solution for
Use with the Immun-Blot®
Horseradish Peroxidase Assay Kit

Catalog Number
170-6535

Store at -20 °C

BIO-RAD

The Horseradish Peroxidase Color Development Reagent, DAB (3,3'-diaminobenzidine), is used for detecting antigens bound to nitrocellulose or other membranes. This substrate develops a brown, insoluble product on the membrane surface after exposure to horseradish peroxidase conjugated antibodies.

Caution: DAB is a suspected carcinogen. Wear gloves, mask, and lab coat when handling. See attached MSDS for further information.

Reagents Available from Bio-Rad

Catalog Number	Product Description	Quantity
170-6515	Affinity Purified Goat Anti-Rabbit IgG (H+L), Human IgG Adsorbed Horseradish Peroxidase Conjugate (GAR-HRP), blotting grade	2 ml
170-6516	Affinity Purified Goat Anti-Mouse IgG (H+L), Human IgG Adsorbed Horseradish Peroxidase Conjugate (GAM-HRP), blotting grade	2 ml

172-1050	Affinity Purified Goat Anti-Human IgG (H+L), Bovine IgG Adsorbed Horseradish Peroxidase Conjugate (GAH-HRP), blotting grade	2 ml
170-6522	Protein A Horseradish Peroxidase Conjugate, blotting grade	1 ml
170-6425	Protein G Horseradish Peroxidase Conjugate, blotting grade	1 ml
161-0307	Biotinylated Standards Kit, HRP, Low Range, contains Biotinylated Standards, Low Range, 250 µl, and Avidin-HRP	2 ml
161-0312	Biotinylated Standards Kit, HRP, High Range, contains Biotinylated Standards, High Range, 250 µl, and Avidin-HRP	2 ml
170-6535	HRP Color Development Reagent, DAB (3,3'-diaminobenzidine)	5 g

Other Reagents Required to Generate the Color Development Solution

1. Tris - Bio-Rad catalog number 161-0716
2. Sodium chloride (NaCl) - ACS reagent grade

3. Hydrogen peroxide - 30% H₂O₂ aqueous solution, stabilized
4. Hydrochloric acid (HCl) - ACS reagent grade

Procedure to Generate the Color Development Solution

1. **Tris buffered saline** (20 mM Tris, 500 mM NaCl, pH 7.5), 2 L: Dissolve 4.84 g Tris and 58.48 g NaCl in ~ 1.5 distilled, deionized H₂O. Adjust to pH 7.5 with HCl. Adjust the volume to 2 L with dd H₂O. Do not use sodium azide in this solution, as it is an inhibitor of horseradish peroxide. If a bacteriostat is necessary, use thimerosal at 0.01% concentration.
2. **DAB color development solution:** Dissolve 50 mg DAB in 100 ml TBS. Add 10 µl 30% H₂O₂. Make this solution just before use.