

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 H<sub>2</sub>O for 10 minutes. Change the water at least once to remove remaining color development solution.

<b>Reagent Storage</b>	<b>Temperature</b>	<b>Shelf Life</b>
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	<b>Affinity Purified Goat Anti-Rabbit IgG (H+L), Human IgG Adsorbed Horseradish Peroxidase Conjugate (GAR-HRP)</b> , blotting grade	2 ml
170-6516	<b>Affinity Purified Goat Anti-Mouse IgG (H+L), Human IgG Adsorbed Horseradish Peroxidase Conjugate (GAM-HRP)</b> , blotting grade	2 ml

172-1050	<b>Affinity Purified Goat Anti-Human IgG (H+L), Bovine IgG Adsorbed Horseradish Peroxidase Conjugate (GAH-HRP)</b> , blotting grade	2 ml
170-6522	<b>Protein A Horseradish Peroxidase Conjugate</b> , blotting grade	1 ml
170-6425	<b>Protein G Horseradish Peroxidase Conjugate</b> , blotting grade	1 ml
161-0307	<b>Biotinylated Standards Kit, HRP, Low Range</b> , contains Biotinylated Standards, Low Range, 250 µl, and Avidin-HRP	2 ml
161-0312	<b>Biotinylated Standards Kit, HRP, High Range</b> , contains Biotinylated Standards, High Range, 250 µl, and Avidin-HRP	2 ml
170-6535	<b>HRP Color Development Reagent, DAB</b> (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% H<sub>2</sub>O<sub>2</sub> 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 H<sub>2</sub>O. Adjust to pH 7.5 with HCl. Adjust the volume to 2 L with dd H<sub>2</sub>O. 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% H<sub>2</sub>O<sub>2</sub>. Make this solution just before use.