DAB Substrate Kit

**Vector® DAB** Substrate (3,3’-diaminobenzidine) produces a brown reaction product in the presence of peroxidase (HRP) enzyme. Adding nickel chloride to the substrate working solution results in a gray-black reaction product.

**Vector® DAB** Substrate can be used on tissue sections or cells, or on membranes such as nitrocellulose, PVDF, or nylon. **Vector® DAB** Substrate is also suitable for darkfield and electron microscopy (EM).

The **Vector® DAB** Substrate Kit contains all of the reagents necessary to prepare either a DAB or a DAB/nickel substrate working solution. These reagents are supplied in convenient dropper bottles.

**REAGENTS:**
- 6 ml Buffer Stock Solution
- 6 ml Hydrogen Peroxide Solution
- 6 ml DAB Stock Solution
- 6 ml Nickel Solution

**STORAGE:**
- Store reagents in original bottles at 2-8 ºC.
- Avoid storing reagents or working solution in strong direct light.

**PREPARATION OF SUBSTRATE WORKING SOLUTION:**
- To 5.0 ml of distilled water*  
  Add 2 drops (approximately 84 µl†) of Buffer Stock Solution  
  Add 4 drops (approximately 100 µl†) of DAB Stock Solution  
  Add 2 drops (approximately 80 µl†) of the Hydrogen Peroxide Solution  
  If a gray-black reaction product is desired  
  Add 2 drops (approximately 80 µl†) of the Nickel Solution  
- Mix well before use

* For blot applications use 15 ml of distilled water.
† Drop volumes differ due to solvent compositions.

**INSTRUCTIONS FOR USE:**

**For Tissues or Cells**

After incubation with a peroxidase (HRP) detection system, rinse well. Incubate with the substrate working solution at room temperature for 2-10 minutes. Optimal development times should be determined by the investigator.

Wash for 5 minutes in water.

Counterstain if desired. (See counterstain compatibility chart on reverse side.) Coverslip with either a non-aqueous mounting medium such as **VectaMount**™ (Cat. No. H-5000) or an aqueous mounting medium such as **VectaMount**™ AQ (Cat. No. H-5501).

**For Blots**

Development time is generally 10-20 minutes at room temperature. When development is satisfactory, rinse membrane in water and air dry.

**NOTES:**

We recommend using glass-distilled water in the preparation of substrate buffer. Deionized water may contain inhibitors of the peroxidase reaction. Solutions containing sodium azide or other inhibitors of peroxidase activity should not be used in diluting the peroxidase substrate.

**Variations in color intensity of the stock and working solutions may be seen between lots of this product. These variations will not affect the product stability or the intensity of the staining.**

The working solution remains stable for up to six hours when stored at 2-8 ºC.

The DAB reaction product can be intensified using a DAB Enhancing Solution (Cat. No. H-2200) after development.

**IMPORTANT:** DAB and nickel chloride are suspected carcinogens. Appropriate care should be exercised when using these reagents including gloves, eye protection, lab coats, and good laboratory procedures. Dispose in accordance with local regulations.