



DAB
Peroxidase Substrate
Cat. No. SK-4100

Store at 2-8 °C

Burlingame, CA USA

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 °C.
- 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^\dagger) of Buffer Stock Solution
 - Add 4 drops (approximately 100 μl^\dagger) of DAB Stock Solution
 - Add 2 drops (approximately 80 μl^\dagger) of the Hydrogen Peroxide Solution

If a gray-black reaction product is desired

Add 2 drops (approximately 80 μl^\dagger) 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 °C.

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.

For Laboratory Use

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