

Vector® VIP Peroxidase Substrate Cat. No. SK-4600

Store at 2-8 °C

Burlingame, CA USA

# Vector® VIP Substrate

**Vector® VIP** Substrate produces a purple reaction product in the presence of peroxidase (HRP) enzyme. **Vector® VIP** Substrate can be used on tissue sections or cells, or on membranes such as nitrocellulose, PVDF, or nylon. **Vector® VIP** Substrate is also suitable for darkfield and electron microscopy (EM).

The **Vector® VIP** Substrate Kit contains all of the reagents (except buffer) necessary to prepare the substrate working solution. These reagents are supplied in convenient dropper bottles.

#### REAGENTS:

7 ml Reagent 1
5 ml Reagent 2
5 ml Reagent 3
7.2 ml Hydrogen Peroxide 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 ml of PBS\* (10 mM sodium phosphate, 0.9% saline, pH 7.5)
  - Add 3 drops (approximately 90  $\mu$ 1<sup>†</sup>) of Reagent 1
  - Add 3 drops (approximately 75  $\mu$ l<sup>†</sup>) of Reagent 2 Add 3 drops (approximately 75  $\mu$ l<sup>†</sup>) of Reagent 3
  - Add 3 drops (approximately  $120 \mu l^{\dagger}$ ) of Hydrogen Peroxide Solution
- · Mix well before use
- \* For blots the buffer volume can be increased to 15ml.

### INSTRUCTIONS FOR USE:

### For Tissues or Cells

After incubation with a peroxidase (HRP) detection system, rinse well. Incubate with the substrate working solution for 2-15 minutes. Optimal development times should be determined by the investigator.

Wash slides for 5 minutes in water.

Counterstain, if desired, with Vector® Hematoxylin (Cat. No. H-3401), Vector® Hematoxylin QS (Cat. No. H-3404), or Vector® Methyl Green (Cat. No. H-3402) (See chart on reverse.) Coverslip with a non-aqueous mounting medium such as *VectaMount*™ (Cat. No. H-5000). Do not mount with aqueous mounting media.

Note: Vector® VIP is not compatible with all methyl green formulations.

#### For Blots

Development time is generally 10 - 20 minutes. When development is satisfactory, wash membrane in water for 5 minutes 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.

The color of the reagent solutions may darken with time. This will have no effect on the quality or intensity of the staining.

Prolonged incubation in alcohol or use of alcohol-based differentiating or bluing solutions may decrease sensitivity.

Do not use recycled alcohol.

**IMPORTANT:** Little is known about the toxicity and carcinogenicity of the substrate kit components. Appropriate care should be exercised when using this reagent including gloves, eye protection, lab coats, and good laboratory procedures. Dispose in accordance with local regulations.

For Laboratory Use 10/13

<sup>†</sup> Drop volumes differ due to solvent compositions.