



## Vector® Black Alkaline Phosphatase Substrate Kit II

### Catalog Number SK-5200

The Vector® Black substrate kit contains all of the reagents necessary (except buffer) to prepare a working solution for either immunocytochemical or membrane-based applications. Vector® Black produces a brown/black reaction product that can be permanently mounted.

#### DISPENSING REAGENTS:

For convenience, the reagents are supplied in dropper bottles. When dispensing drops, hold the bottle in an inverted vertical position and squeeze gently. To prevent evaporation, secure the opaque caps on the bottles when they are not in use. **DO NOT PIPET REAGENTS DIRECTLY FROM BOTTLES.** Drop volumes of each component may be different due to solvent characteristics. Proper concentrations of substrate components in the working solution are assured only by using the drop dispensers.

#### INSTRUCTIONS FOR USE:

Prepare the Vector® Black substrate working solution immediately before use.

1. To 5 mls\* of 100 mM Tris-HCl, pH 9.5 buffer, add 2 drops of Reagent 1. Mix well.
2. Add 2 drops of Reagent 2. Mix well.
3. Add 2 drops of Reagent 3. Mix well.

\* For some applications using nitrocellulose or nylon membranes, the buffer volume can be increased to 15 ml.

Incubate tissue sections or membranes with substrate solution at room temperature until suitable staining develops. Development times should be determined by the investigator but generally 20-30 minutes provides good staining intensity. Improved staining may be obtained by developing the substrate in the dark.

Wash sections in assay buffer for 5 minutes.

For permanent mounting: Rinse in tap water, counterstain, if desired (see chart on reverse), dehydrate, clear and mount in a permanent mounting medium, such as **VectaMount™**, Catalog No. H-5000.

#### NOTES:

1. **IMPORTANT.** For tissue, using 0.1% Tween® 20 in the Vector® Black working solution may increase the sensitivity and crispness of staining especially when non-enzymatic antigen retrieval methods are used in the immunostaining protocol. Tween® 20 should not be added to the Vector® Black substrate solution for membrane applications.
2. Vector® Black reaction product should appear brown/black. Bluish staining indicates that the concentration of the primary antibody is too high and should be decreased.
3. Prolonged incubation in the substrate working solution tends to increase background toning.
4. Do not heat Vector® Black substrate kit components or working solution. Heating decreases staining intensity.
5. The reagents should be stored at 4 °C and protected from light whenever possible. Occasionally a precipitate may form in some reagents upon prolonged storage. This will have no effect on the quality or intensity of the staining. Do not filter the reagents or working solution.
6. When using neural tissue, Vector® Black is not recommended for visualizing neural processes, fibers, or terminals (inadequate staining may occur).
7. Endogenous alkaline phosphatase activity (other than the intestinal isoenzyme) can be inhibited by the addition of levamisole (Cat. No. SP-5000) to the buffer prior to the preparation of the working solution. Alternatively, endogenous alkaline phosphatase, including the intestinal isoenzymes, can be inactivated before immunostaining with the use of **BLOXALL™** Blocking Solution (Cat. No. SP-6000) or with other tissue pretreatments (Bulman AS and Heyderman E; J. Clin. Pathol. 34, 1349-1351, 1981).

**IMPORTANT:** Little is known about the toxicity and carcinogenicity of the substrate components. Care should be taken in the handling and disposing of all the reagents.