



Vector® Blue Alkaline Phosphatase Substrate Kit III

Cat. No. SK-5300

The Vector® Blue substrate kit contains all of the reagents necessary (except buffer) to prepare a working solution for immunocytochemical applications. Vector® Blue produces a blue reaction product that can be aqueously or 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® Blue substrate working solution immediately before use in the mixing bottle or test tube.

1. To 5 ml of 100 mM Tris-HCl, pH 8.2 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.

Incubate tissue sections 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 buffer for 5 minutes. Rinse slides in tap water, and counterstain, if desired (see chart on reverse).

* For permanent mounting: Vector® Blue is partially soluble in xylene; avoid xylene-based clearing agents and mounting media. Dehydrate sections, clear in a xylene-substitute clearing agent such as HistoClear™ or Clear-Rite 3 and mount in a non-xylene-based mounting media, such as **VectaMount™** (Cat. No. H-5000).

For aqueous mounting: Use an aqueous-based mounting medium such as **VectaMount™ AQ** (Cat. No. H-5501).

NOTES:

1. IMPORTANT. Using 0.1% Tween® 20 in the Vector® Blue working solution may increase the sensitivity and crispness of staining especially when non-enzymatic antigen retrieval methods are used in the immunostaining protocol.
2. Increasing the incubation time in substrate solution up to four hours may increase staining sensitivity 2-4 times, however background toning may also increase.
3. Do not heat Vector® Blue substrate kit components or working solution. Heating decreases staining intensity.
4. 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.
5. Do not put sodium azide in the buffers used for the Vector® Blue working solution; it will prevent staining.
6. When using neural tissue, Vector® Blue is not recommended for visualizing 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. Intestinal alkaline phosphatase activity can be inhibited, before immunostaining, with several 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.

*Tween® 20 is a trademark of Atlas Chemical Industries
HistoClear™ is a trademark of National Diagnostics*

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