



**Vector® Blue
Alkaline Phosphatase
Substrate**
Cat. No. SK-5300

Store at 2-8 °C

Burlingame, CA USA

Vector® Blue Substrate

Vector® Blue Substrate produces a blue reaction product in the presence of alkaline phosphatase (AP) enzyme. **Vector® Blue** Substrate can be used on tissue sections or cells, or on membranes, such as nitrocellulose, PVDF, or nylon.

The **Vector® Blue** 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:

4 ml Reagent 1
4 ml Reagent 2
3 ml Reagent 3

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 100 mM - 200 mM Tris-HCl, pH 8.2 - 8.5 buffer*
 - Add 2 drops (80 μ l) of Reagent 1
 - Add 2 drops (80 μ l) of Reagent 2
 - Add 2 drops (45 μ l) of Reagent 3
- Mix well before use

* 0.1% Tween® 20 in the Vector® Blue working solution is recommended, especially for use with antigen retrieved tissues.

INSTRUCTIONS FOR USE:

For Tissues or Cells

After incubation with an AP detection system, rinse well. Incubate, with the substrate working solution for 20-30 minutes. Optimal development times should be determined by the investigator. Improved staining may be obtained by developing the substrate in the dark.

Wash slides in buffer for 5 minutes. Rinse in 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™** (Catalog No. H-5000).

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

For Blots

Incubate blot for 20-30 minutes. When development is satisfactory, rinse membrane with water and air dry.

NOTES:

1. Increasing the incubation time in substrate solution up to four hours may increase staining sensitivity 2-4 times, however background toning may also increase.
2. Do not put sodium azide in the buffer used for the **Vector® Blue** working solution.
3. Do not heat **Vector® Blue** substrate kit components or working solution.
4. 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. 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 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.

Tween® 20 is a registered trademark of Atlas Chemical Industries

HistoClear™ is a trademark of National Diagnostics

Clear-Rite™ 3 is a trademark of Richard Allan Scientific

For Laboratory Use

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