

# Vector® Blue

## Alkaline Phosphatase Substrate

<b>Cat. No.</b>	<b>SK-5300</b>
<b>Storage</b>	Store reagents in original bottles at 2-8 °C. Avoid storing reagents or working solution in strong direct light.
<b>Description</b>	This kit contains all of the reagents necessary to prepare the substrate working solution. Vector Blue Substrate produces a blue reaction product in the presence of alkaline phosphatase (AP) enzyme.

### Components

Product Name	Volume
Vector Blue Reagent 1	4 ml
Vector Blue Reagent 2	4 ml
Vector Blue Reagent 3	3 ml

### Preparation of Substrate Working Solution

To 5 ml of 100 mM - 200 mM Tris-HCl, pH 8.2 - 8.5 buffer\*:

- Add 2 drops ( $\approx 80 \mu\text{l}^\dagger$ ) of Vector Blue Reagent 1
- Add 2 drops ( $\approx 80 \mu\text{l}^\dagger$ ) of Vector Blue Reagent 2
- Add 2 drops ( $\approx 45 \mu\text{l}^\dagger$ ) of Vector Blue Reagent 3

Mix well before use. Use immediately.

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

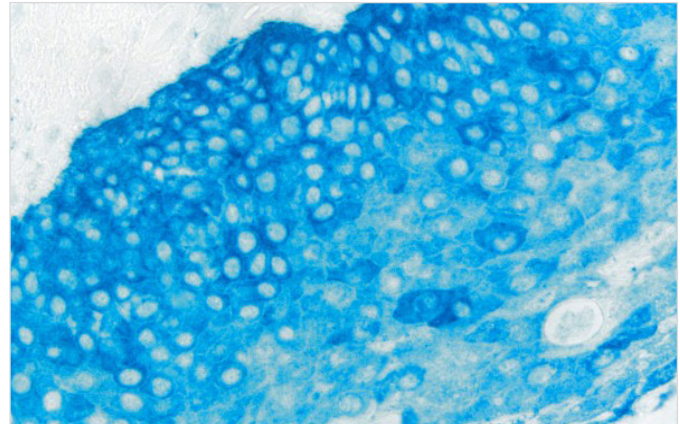
† Drop volumes differ due to solvent compositions.

**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.

### Instructions for Use

After incubation with an AP detection system, rinse sections in buffer. 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.



*Tonsil: Cytokeratin AE1/AE3 (m) detected with ImmPRESS®-AP Reagent and Vector Blue AP Substrate (blue). No Counterstain.*

For permanent, non-aqueous 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 Histo-Clear® or Clear-Rite 3™ and coverslip in a non-xylene-based, non-aqueous mounting media, such as VectaMount® Mounting Medium (H-5000) or VectaMount® Express Mounting Medium (H-5700).

For aqueous mounting: Coverslip using an aqueous mounting media such as VectaMount® AQ Mounting Medium (H-5501).

### Notes

Increasing the incubation time in substrate solution up to four hours may increase staining sensitivity 2-4 times, however background toning may also increase.

Detailed product listings, specifications, protocols and additional information is available on our website: [vectorlabs.com](http://vectorlabs.com)