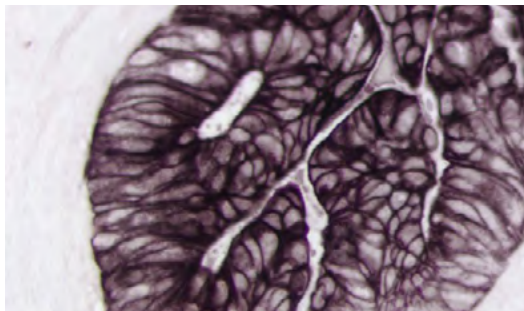


Vector® Black

Alkaline Phosphatase Substrate

Cat. No.: SK-5200

Storage: 2-8 °C



Colon Carcinoma: Pan-Cytokeratin (m) detected with VECTASTAIN® ABC-AP Kit and Vector Black Substrate (brown-black). No Counterstain.

DESCRIPTION

This kit contains all of the reagents necessary to prepare the substrate working solution. Vector Black Substrate produces a brown-black reaction product in the presence of alkaline phosphatase (AP) enzyme.

COMPONENTS

<u>Product Name</u>	<u>Volume</u>
Vector Black Reagent 1	4 ml
Vector Black Reagent 2	4 ml
Vector Black Reagent 3	3 ml
Substrate Mixing Bottle	(Empty)

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 Tris-HCl, pH 9.5 buffer *

- Add 2 drops (≈80 µl†) of Vector Black Reagent 1
- Add 2 drops (≈84 µl†) of Vector Black Reagent 2
- Add 2 drops (≈40 µl†) of Vector Black Reagent 3

Mix well before use. Use immediately.

* 0.1% Tween 20 in the Vector Black working solution is recommended 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 with buffer for 5 minutes. Rinse in water and counterstain, if desired (see chart on reverse).

For permanent, non-aqueous mounting: Dehydrate, clear and coverslip using a non-aqueous mounting media, such as VectaMount® Mounting Medium (H-5000).

NOTES

1. 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.
2. Prolonged incubation in the substrate working solution can increase background toning in tissue sections.

Detailed product listings, specifications, protocols and additional information is available on our website: vectorlabs.com