

# VECTASTAIN® ABC-AP Kit

## Alkaline Phosphatase (AP)

<b>Cat. No.</b>	<b>AK-5000, AK-5001, AK-5002, AK-5004</b>
<b>Storage</b>	Store reagents in original bottles at 2-8 °C. Do not freeze.
<b>Description</b>	<p>Instructions for Immunohistochemical staining.</p> <p>VECTASTAIN ABC-AP Kits are enzymatic, avidin/biotin based amplification kits that produces crisp, highly sensitive, specific staining with low background.</p> <p>The reagents are supplied in convenient dropper bottles. (To remove the drop dispenser tip, press laterally with thumb until tip snaps off).</p>

### Kit Components

Product Name	Volume
Blocking Serum (Normal Serum) - yellow-labeled bottle	3 ml
Biotinylated Secondary Antibody - blue-labeled bottle	1 ml
Reagent A (Avidin, ABC-AP) - gray-labeled bottle	2 ml
Reagent B (Biotinylated AP, ABC-AP) - gray-labeled bottle	2 ml

The VECTASTAIN ABC-AP Kit will stain approximately 1000-2000 sections. Note: The VECTASTAIN ABC-AP Kit (Standard), AK-5000 contains only Reagent A and Reagent B.

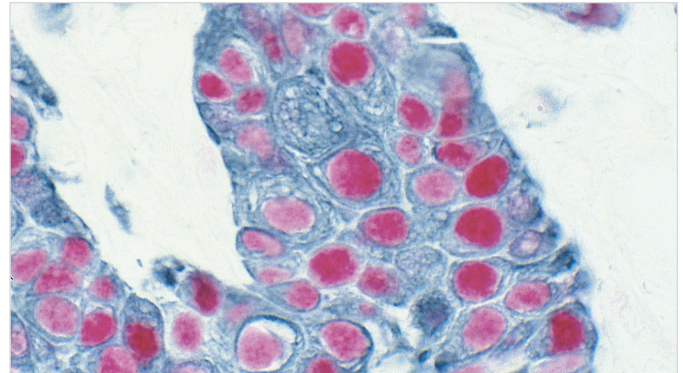
### Preparation of Vectastain Working Solutions

A number of different buffers can be used in the VECTASTAIN ABC-AP system. One of the most common is 10 mM sodium phosphate, pH 7.5, 0.9% saline (PBS). The VECTASTAIN working solutions are prepared as follows:

- **Blocking Serum (Normal Serum):** add three (3) drops (150 µl) of stock (yellow label) to 10 ml of buffer. The preferred serum for blocking is prepared from the same animal species in which the biotinylated secondary antibody is made.
- **Biotinylated Antibody:** add one (1) drop (50 µl) of normal blocking serum stock (yellow label) to 10 ml buffer and then add one (1) drop (50 µl) of biotinylated secondary antibody stock (blue label).
- **VECTASTAIN ABC-AP Reagent:** add two (2) drops (100 µl) of Reagent A (gray label) to 10 ml of buffer. Then add two (2) drops (100 µl) of Reagent B (gray label) to the same container, mix immediately, and allow VECTASTAIN® Reagent to stand for about 30 minutes before use.

### Staining Procedure

1. For paraffin sections, deparaffinize and hydrate through xylenes or other clearing agents and graded alcohol series.



*Tumor - Double Label: p53 protein (m), VECTASTAIN ABC-AP Kit, Vector Red AP Substrate (red), Pan-Cytokeratin (sheep), VECTASTAIN Elite® ABC Kit, Vector SG HRP Substrate (blue/gray)*

- For frozen sections or cell preparations fix with acetone or an appropriate fixative for the antigen under study, if necessary. Wash for 5 minutes in tap water.
2. If antigen unmasking is required, perform this procedure using a Vector® Antigen Unmasking Solution, Citrate-based, pH 6.0 (H-3300) or Tris-based pH 9.0 (H-3301).
3. If quenching of endogenous alkaline phosphatase activity is required, incubate the slides in BLOXALL® Blocking Solution for 10 minutes.
4. Wash in buffer for 5 minutes.
5. Incubate for 20 minutes with normal blocking serum.
6. Tip off excess serum from sections.
7. Incubate for 30 minutes with primary antibody diluted in appropriate diluent such as buffer with 2.5 % normal serum or R.T.U. Animal-Free Blocker® and Diluent (SP-5035).
8. Wash for 5 minutes in buffer.
9. Incubate for 30 minutes with diluted biotinylated secondary antibody.
10. Wash for 5 minutes in buffer.
11. Incubate for 30 minutes with prepared VECTASTAIN ABC-AP Reagent.
12. Wash for 5 minutes in buffer.
13. Incubate section for 20-30 minutes in AP substrate solution (not included in kit) until desired stain intensity develops.
14. Rinse in tap water.
15. Counterstain (optional), clear and mount.

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