USER GUIDE

# VECTASTAIN® Elite® ABC-HRP Kit

## Peroxidase

Together we breakthrough™

**Cat. No.** PK-6100, PK-6101, PK-6102, PK-6103, PK-6104, PK-6105, PK-6106

**Storage** Store reagents in original bottles at 2–8 °C. Do not freeze.

**Description** Instructions for Immunohistochemical staining.

The VECTASTAIN Elite ABC Kit is an enzymatic, avidin/biotin based amplification system that produces crisp, highly sensitive, specific staining with low background.

The reagents are supplied in convenient dropper bottles. (To remove the drop dispenser tip, press laterally with thumb until tip snaps off).

**Kit Components**

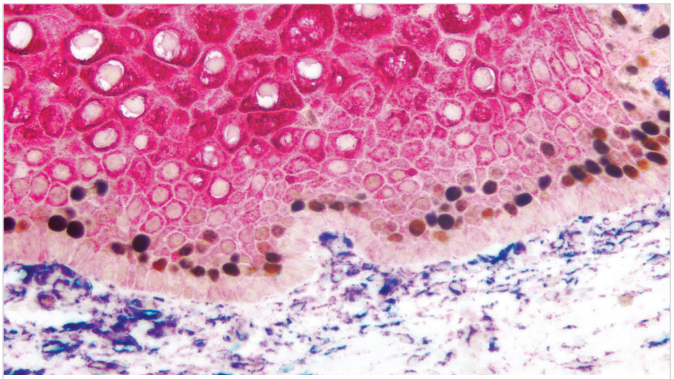
| Product | Volume |
|---|---|
| Blocking Serum (Normal Serum) – yellow-labeled bottle | 3 ml |
| Biotinylated Secondary Antibody – blue-labeled bottle | 2 ml |
| Reagent A (Avidin, ABC Elite) – gray-labeled bottle | 2 ml |
| Reagent B (Biotinylated HRP, ABC Elite) – gray-labeled bottle | 2 ml |

The VECTASTAIN Elite ABC Kit will stain approximately 500–1000 sections. Note: The VECTASTAIN Elite ABC Kit (Standard), PK-6100 contains only Reagent A and Reagent B.

### Preparation of VECTASTAIN Working Solutions

A number of different buffers can be used in the VECTASTAIN Elite ABC system. One of the most common is 10 mM sodium phosphate, pH 7.5, 0.9% saline (PBS). Do not use sodium azide when preparing buffers. 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 three (3) drops (150 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 Elite ABC Reagent: add two (2) drops (100 µl) of Reagent A (gray label) to 5 ml of buffer. Then add two (2) drops(100 µl) of Reagent B (gray label) to the same container, mix immediately, and allow VECTASTAIN Elite ABC Reagent to stand for 15–30 minutes before use.



*Tumor – Triple Label: Ki67 (m), VECTASTAIN Elite ABC Kit, DAB Substrate (brown), CD34 (m), VECTASTAIN ABC-AP Kit, Vector Blue Substrate (blue, Cytokeratin AE1/AE3 (m), VECTASTAIN ABC-AP Kit, Vector Red Substrate (red)*

### Staining Procedure

1. For paraffin sections, deparaffinize and hydrate through xylenes or other clearing agents and graded alcohol series.
   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 peroxidase activity is required, incubate the slides in BLOXALL® Blocking Solution (SP-6000) for 10 minutes.
4. Wash in buffer for 5 minutes.
5. Incubate for 20 minutes with diluted 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 Elite ABC Reagent.
12. Wash for 5 minutes in buffer.
13. Incubate in a peroxidase 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

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