

VECTASTAIN® Elite® ABC-HRP Kit

Peroxidase (Universal)

Cat. No.	PK-6200
Storage	Store reagents in original bottles at 2-8 °C. Do not freeze.
Description	Instructions for Immunohistochemical staining.

The 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.

Kit Components

Product	Volume
Normal Horse Serum - yellow-labeled bottle	3 ml
Horse Anti-Mouse/Rabbit IgG, Biotinylated - 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 Universal Kit will stain approximately 500-1000 sections.

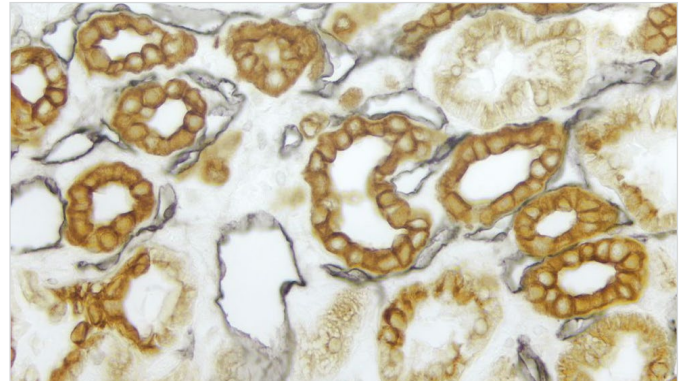
Preparation of VECTASTAIN Working Solutions

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, 0.15M NaCl (PBS). Do not use sodium azide when preparing buffers. The VECTASTAIN working solutions are prepared as follows:

- **Blocking Serum (Normal Serum):** add one (1) drop (50 µl) of stock (yellow label) to 5 ml of buffer. The preferred serum for blocking is prepared from the same animal species in which the biotinylated secondary antibody is made.
- **Biotinylated Universal Antibody:** add two (2) drops (100 µl) of normal blocking serum stock (yellow label) to 5 ml buffer and then add two (2) drops (100 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.

Staining Procedure

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



Kidney – Double Label: CD34 (m), VECTASTAIN Elite ABC Universal Kit, DAB-Ni Substrate (gray/black), Cytokeratin 8/18 (m), VECTASTAIN Elite ABC Universal Kit, DAB Substrate (brown)

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 prepared Normal Serum.
6. Tip off excess serum from sections.
7. Incubate for 30 minutes with primary antibody diluted in buffer with 2.5 % normal serum.
8. Wash for 5 minutes in buffer.
9. Incubate for 30 minutes with prepared Biotinylated Universal 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