VECTOR LABORATORIES

part of Maravai LifeSciences

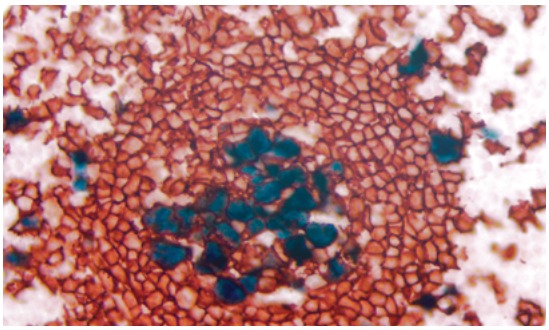
USER GUIDE

VECTASTAIN® ABC-AP Universal Kit
Alkaline Phosphatase (Horse Anti-Mouse/Rabbit IgG)

Cat. No.: AK-5200

Storage: 2-8 °C

Instructions for Immunohistochemical staining.



Tumor - Double Label:
• Cyclin A (m), VECTASTAIN ABC-AP Universal Kit, Vector Blue AP Substrate (blue) • CD2- (m), VECTASTAIN Elite ABC Universal Kit, Vector NovaRED HRP Substrate (red)

DESCRIPTION

VECTASTAIN ABC-AP Kits are enzymatic, avidin/biotin based amplification kits that produces crisp, highly sensitive, specific staining with low background.

The reagents are supplied in convenient dropper bottles.

KIT COMPONENTS

Product Name	Volume
Normal Horse Serum – yellow-labeled bottle	3 ml
Horse Anti-Mouse/Rabbit IgG, Biotinylated – blue-labeled bottle	2 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 Universal Kit will stain approximately 1000-2000 sections.

STORAGE:

- Store reagents in original bottles at 2-8 °C
- Do not freeze.

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 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) drop (100 µl) of biotinylated universal secondary antibody stock (blue label).
- VECTASTAIN ABC-AP Reagent: add one (1) drop (50 µl) of Reagent A (gray label) to 5 ml of buffer. Then add one (1) drop (50 µl) of Reagent B (gray label) to the same container, mix immediately, and allow VECTASTAIN Reagent to stand for about 15-30 minutes before use.

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 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 diluted normal blocking serum.
6. Tip off excess serum from sections.
7. Incubate for 30 minutes with primary antibody (mouse or rabbit) diluted in buffer with 2.5 % normal serum.
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 substate 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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