INTRODUCTION

The VECTASTAIN® Elite ABC Kit is widely accepted as one of the most sensitive, economical and reliable immunoperoxidase detection systems available. The enhanced sensitivity of the VECTASTAIN® Elite ABC Kit is particularly important in the localization of antigens present in low amounts or in cases where the cost of the primary antibodies is significant. The increased sensitivity also provides an option to substantially reduce staining times.

The advanced avidin/biotin technology of the VECTASTAIN® Elite ABC system results in an ABC complex that is smaller, very uniform, and highly active. This allows more access for binding to a biotinylated target. As with all VECTASTAIN® ABC systems, the complex is formed by mixing optimized formulations of two paired reagents: Reactant A (Avidin DH, an avidin that is modified using a proprietary process to eliminate non-specific binding) and Reactant B (Biotinylated peroxidase H with enhanced enzyme activity). The two important properties of avidin - an extraordinarily high affinity for biotin (over one million times higher than an antibody for most antigens), and four biotin-binding sites - in addition to proprietary technology, allow optimal macromolecular complexes to be formed. The complexes remain stable for many hours after formation.

The VECTASTAIN® Elite ABC Reagent can be used to detect any molecule that is biotinylated. This property gives the ABC method great versatility in the types of targets that can be detected as well as the types of applications in which it can be employed. Biotinylated primary antibodies, secondarys, lectins, neuronal tracers, nucleic acids, and ligands can be effectively visualized in applications such as:

- Tissue and cell staining
- Protein and nucleic acid blot detection
- In situ hybridization detection
- Enzyme immunoassays
- Neuronal tracing

With the exception of the “Standard Kit” which includes Reactant A and Reactant B only, the VECTASTAIN® Elite ABC Kits are configured with Reactant A, Reactant B, a biotinylated, affinity-purified secondary antibody and matching normal blocking serum. The secondary antibodies are conjugated to ensure the maximum degree of labeling without compromising the specificity or affinity of the antibody. Due to the versatility of the avidin/biotin interaction, the VECTASTAIN® Elite ABC Kit is modular, and along with our selection of secondary antibodies, can accommodate a wide array of primary antibody and tissue species.

INSTRUCTIONS FOR IMMUNOHISTOCHEMICAL STAINING

**COMPONENTS**

**Reagents supplied:**
- Blocking Serum (Normal Serum) in yellow-labeled small bottle - 3 ml
- Biotinylated, affinity-purified anti-immunoglobulin in blue-labeled small bottle - 1 ml
- Reactant A (Avidin DH) in gray-labeled small bottle - 2 ml
- Reactant B (Biotinylated Horseradish Peroxidase H) in gray-labeled small bottle - 2 ml

The VECTASTAIN® Elite ABC Kit contains sufficient reagents to stain approximately 500-1000 tissue sections.

**NOTE:** The VECTASTAIN® Elite ABC Kit (Standard), PK-6100, contains only Reactant A and Reactant B.

**Storage:**
Stock VECTASTAIN® Elite ABC Kit reagents should be stored at 2-8 °C.

**Reagents not supplied:**
- Primary Antibody
- Buffer
- Hydrogen Peroxide
- Oxidizable Peroxidase Substrate

**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). 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 in mixing bottle (yellow label). The preferred serum for blocking is prepared from the same 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 in mixing bottle and then add one (1) drop (50 µl) of biotinylated antibody stock (blue label).
- VECTASTAIN® Elite ABC Reagent: add exactly two (2) drops (100 µl) of REAGENT A (gray label) to 5 ml of buffer in the ABC Reagent large mixing bottle. Then add exactly two (2) drops (100 µl) of REAGENT B (gray label) to the same mixing bottle, mix immediately, and allow VECTASTAIN® Elite ABC Reagent to stand for about 30 minutes before use.

**NOTE:** After completion of the staining procedure discard dilute working solutions, and rinse the containers with distilled water.

For convenience, VECTASTAIN® Elite ABC Kits include mixing bottles to prepare working solutions of reagents. As supplied, the drop dispenser tip is in an inverted position and is not inserted into the bottle. After the buffer and appropriate reagents are added to the bottle, insert the drop dispenser tip into the white or gray opaque cap in correct orientation. Place the entire unit onto the bottle and twist on the cap. As the cap is tightened, the drop dispenser tip will snap into place. To remove the drop dispenser tip for refilling, merely press laterally with thumb until the tip snaps off. When dispensing drops, hold the bottle in an inverted vertical position and squeeze gently. To prevent evaporation, secure the opaque white or gray caps on the bottles when they are not in use.

**RAPID STAINING PROCEDURE**

The sensitivity of the VECTASTAIN® Elite ABC Kit permits development of shortened immunoperoxidase staining protocols. In this section some guidelines are provided for a rapid staining method having a sensitivity and staining quality equivalent to the full-length VECTASTAIN® Elite ABC protocol.

1. Prepare paraffin-embedded or frozen sections for staining as described elsewhere. Prepare VECTASTAIN® Elite ABC Kit reagents as follows: For the Biotinylated Antibody, add one drop concentrated stock to 5 ml of PBS containing 1.5% normal serum. If background staining is a problem, increase the concentration of normal serum up to 10%. For the VECTASTAIN® Elite ABC Reagent, add two drops of Reagent A to 2.5 ml buffer, mix, and then add two drops of Reagent B. Mix and allow to stand for 5-30 minutes before use.
2. If quenching of endogenous peroxidase is required, an accelerated quenching procedure can be employed. Treat sections with 3% hydrogen peroxide in water for 3-5 minutes. Alternatively, incubate in BLOXALL™ Blocking Solution (SP-6000) for 10 minutes.
3. Wash gently with a stream of buffer from a wash bottle.
4. If background staining is a problem, incubate sections for 5-10 minutes in 2%-10% normal serum in buffer.
5. Incubate with primary antibody.
6. Wash as in step 3.
7. Incubate for 10 minutes with diluted biotinylated secondary antibody.
8. Wash as in step 3.
9. Incubate for 5 minutes with VECTASTAIN® ABC Reagent.
10. Wash as in step 3.
11. Incubate in peroxidase substrate solution until desired stain intensity develops.
12. Wash as in step 3.
13. Counterstain, clear and mount.

NOTE: A very rapid procedure that provides excellent staining results can also be performed. Prepare diluted biotinylated secondary antibody 1 drop/ 2.5 ml. Prepare VECTASTAIN® ABC Reagent as in the above protocol. Apply diluted VECTASTAIN® ABC Kit reagents preheated to 37 °C. Incubate sections in each reagent for 2 minutes.

A VECTASTAIN® Universal Quick Kit (PK-8800), based on a preformed streptavidin/peroxidase complex, is also available to perform rapid immunohistochemical staining.

Related Reagents

Antigen Unmasking Solution (dilutes to 25 liters)  
Citrabased, pH 6.0 250 ml H-3300
Tris-based, pH 9.0 250 ml H-3301
Avi n /Biotin Blocking Kit 1 Kit SP-2001
BLOXALL® Blocking Solution 100 ml SP-6000
Imprint™ Hydrophilic Barrier Pen 2- pen set H-4000
Imprint™ Histology Pen 5- pen set H-6100
Vectabond™ Reagent (dilutes to 350 ml) 7 ml SP-1800
VectaMount™ Mounting Medium 60 ml H-5000
VectaMount™ Aq Mounting Medium 60 ml H-5501
Vector® Hematoxylin 500 ml H-3401
Vector® Hematoxylin Q5 100 ml H-3404
Vector® Methyl Green 500 ml H-3402
Vector® Methyl Red 500 ml H-3403
Heat-treated, ultrafiltered normal serum  20 ml S-1000
Normal Goat Serum  2.5% Normal Goat Serum 20 ml S-1012
Normal Horse Serum  2.5% Normal Horse Serum 20 ml S-2000
Normal Chicken Serum  20 ml S-3000
Normal Swine Serum  20 ml S-4000
Normal Rabbit Serum  20 ml S-5000

Detailed product listings, specifications and protocols are available on our website: www.vectorlabs.com

Biotinylated Antibodies Available

The following biotinylated antibodies can be used in conjunction with any VECTASTAIN® ABC Kit.

Biotinylated Anti-Cat IgG (H + L)  1.5 mg BA-9000
made in goat

Biotinylated Anti-Chicken IgG (H + L)  1.5 mg BA-9001
made in goat

Biotinylated Anti-Goat IgG (H + L)  1.5 mg BA-5000
made in rabbit a* 1.5 mg BA-9500
made in horse a* 1.5 mg BA-9500
made in goat d

Biotinylated Anti-Human IgG (H + L)  1.5 mg BA-3000
made in goat d

Biotinylated Anti-Mouse IgG (H + L)  1.5 mg BA-2000
made in horse d 1.5 mg BA-9200
made in goat d 0.5 mg BA-2001

Biotinylated Anti-Rabbit IgG (H + L)  1.5 mg BA-1000
made in goat d

Biotinylated “Universal” Anti-Mouse/Rabbit IgG (H + L) made in horse d 2.1 mg BA-1400

Biotinylated “Universal” Pan-Specific Anti-Mouse/Rabbit/Goat IgG (H + L) made in horse d 2.2 ml BA-1300

*: a - Suitable for use with bovine IgG primary antibodies.
   b - Designed for use in rat tissues.
   c - Designed for use in mouse tissues.
   d - Antibodies included in VECTASTAIN® Elite ABC Kits
   e - Universal Anti-Mouse/Rabbit IgG (BA-1900) should be reconstituted with 2 ml water and diluted 1:50 for use.
   f - Antibody used in the VECTASTAIN® Universal Quick Kits.

†: Chain-specific antibodies are also available.

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