VECTOR LABORATORIES

VECTASTAIN® UNIVERSAL Elite® ABC KIT

(Anti-Mouse IgG/Rabbit IgG)

Cat. No. PK-6200

INSTRUCTIONS FOR IMMUNOHISTOCHEMICAL STAINING

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 Elite ABC complex that is smaller, uniform, and highly active. This allows more accessibility for binding to a biotinylated target. As with all VECTASTAIN® ABC systems, the complex is formed by mixing optimized formulations of two paired reagents: Reagent A (Avidin DH, an avidin that is modified using a proprietary process to eliminate non-specific binding) and Reagent 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 Elite 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, secondaries, 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 Reagent A and Reagent B only, the VECTASTAIN® Elite ABC Kits are configured with Reagent A, Reagent 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.

Vector Laboratories, Inc.• 30 Ingold Road • Burlingame, CA 94010 USA
Tel: (650) 697-3600 • Fax: (650) 697-0339
Email: vector@vectorlabs.com • Website: www.vectorlabs.com

COMPONENTS

Reagents supplied:

- Blocking Serum (Normal Horse Serum) in yellow-labeled small bottle - 3 ml
- Biotinylated, Universal (Anti-Mouse IgG/Rabbit IgG) Antibody in blue-labeled small bottle - 2 ml
- Reagent A (Avidin DH) in gray-labeled small bottle - 2 ml
- Reagent 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), Cat. No. PK-6100, contains only Reagent A and Reagent B.

Storage:

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

Reagents not supplied:

- Primary Antibody
- Buffer
- Hydrogen Peroxide
- 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 Horse Serum): add one (1) drop (50 µl) of stock (yellow label) to 5 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 Universal (Anti-Mouse IgG/Rabbit IgG) Antibody: add two (2) drops (100 µl) of normal blocking serum stock (yellow label) to 5 ml buffer in mixing bottle and then add two (2) drops (100 µl) of biotinylated antibody stock (blue label).
- VECTASTAIN® Elite ABC Reagent: add two (2) drops (100 µl) of REAGENT A (gray label) to 5 ml of buffer in the ABC Reagent large mixing bottle. Then add 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.

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 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.

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

If the reagents are to be diluted beyond their recommended concentrations, first prepare the diluted biotinylated antibody and VECTASTAIN® Elite ABC reagent as described in the instructions. Subsequent dilutions should be made in a buffer containing 0.1% immunohistochemical grade Bovine Serum Albumin (SP-5050), as other preparations can contain undesired impurities. Dilution of these reagents may require longer incubation times and/or higher incubation temperatures to achieve maximum sensitivities.

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 (H-3300) or High pH-based (H-3301).

3. If quenching of endogenous peroxidase activity is required, incubate the slides in **BLOXALL**™ Blocking Solution (SP-6000) for 10 minutes. If endogenous peroxidase activity does not present a problem, this step may be omitted. For alternative quenching procedures please see Note 3.

4. Wash in buffer for 5 minutes.

5. Incubate for 20 minutes with diluted normal blocking serum. (In cases where non-specific staining is not a problem, steps 5 and 6 can be omitted).*

6. Blot excess serum from sections.

7. Incubate for 30 minutes with primary antibody diluted in buffer (see Note 4).

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 VECTASTAIN® Elite ABC Reagent.

12. Wash for 5 minutes in buffer.

13. Incubate in peroxidase substrate solution until desired stain intensity develops. For a list of peroxidase substrates, see “Peroxidase Substrates” (see reverse).

14. Rinse sections in tap water.

15. Counterstain, clear and mount.

* If unwanted staining occurs in the absence of biotinylated secondary antibody, endogenous protein-associated biotin may be present in the tissue. To eliminate this unwanted staining, use an Avidin/Biotin blocking step (SP-2001) between steps 4 and 5.

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 two drops concentrated stock to 2.5 ml of PBS containing 2 drops 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, 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 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 sections with primary antibody.†

6. Wash as in step 3.
7. Incubate sections for 10 minutes with diluted biotinylated secondary antibody.
8. Wash as in step 3.
9. Incubate sections for 5 minutes with VECTASTAIN® Elite ABC Reagent.
10. Wash as in step 3.
11. Incubate sections in peroxidase substrate solution until desired stain intensity develops.
12. Wash as in step 3.
13. Counterstain, clear and mount.

† The concentration, staining time and temperature of the primary antibody should be tailored to an investigator's particular requirements. The increased sensitivity of the VECTASTAIN® Elite ABC Kit allows shorter primary antibody incubation times. For example, at primary antibody concentrations optimal for the regular VECTASTAIN® ABC Kit, incubation times can be reduced at least in half when using the VECTASTAIN® Elite ABC Kit. Higher concentrations of primary antibody allow even shorter incubation times.

NOTE: A very rapid procedure that provides excellent staining results can also be performed. Prepare diluted biotinylated secondary antibody: 4 drops/2.5 ml plus 2 drops normal serum. Prepare VECTASTAIN® Elite ABC Reagent as in the above protocol. Apply diluted VECTASTAIN® Elite 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.

NOTES:

1. VECTASTAIN® Elite ABC Kits can be used in multiple antigen labeling applications. A brochure with protocols is available - "Discovery Through Color". Please request a free printed copy or download it from our website: www.vectorlabs.com. Additional information on Enzyme Substrate Combinations, Counterstain/Substrate Compatibility, and Relative Substrate Sensitivity is also available on our website.
2. Solutions containing sodium azide or other inhibitors of peroxidase activity should not be used in diluting the peroxidase substrate or the VECTASTAIN® Elite ABC Reagent. Do not add normal serum, non-fat dried milk, culture media or other potential sources of biotin to the ABC reagent. This may result in reduced sensitivity.
3. Alternative peroxidase quenching procedures:

   For formalin fixed cells and tissues, incubate in 3% H_2O_2 in tap water for 5 minutes or 0.3% H_2O_2 in either methanol or water for 30 minutes.

   For frozen tissue or cell preparations, use 0.3% H_2O_2 in 0.3% normal serum in PBS for 5 minutes, or 0.3% H_2O_2 in methanol for 30 minutes or use other published methods. (eg. Andrew, S.M., Jasani, B., Histochem J. 1987, 19, 426-30).
4. To avoid adsorption of the antibody to the plastic or glass container in which the final dilution is made, the primary antibody may be diluted in buffers containing diluted (2.5%) normal serum. Alternatively, 0.1% immunohistochemical grade Bovine Serum Albumin (SP-5050) can be used. Other grades of BSA can contain undesired impurities.
5. Use only freshly prepared buffers. Bacterial contamination which can occur in buffers stored at room temperature may affect the quality of the staining. It is recommended that the VECTASTAIN® Elite ABC Reagent and substrate solution be prepared with glass distilled water. Deionized water (even with low conductivities) may contain inhibitors of peroxidase and can reduce sensitivity.
6. The A and B reagents in the kits are matched. Do not use an A reagent from one kit with a B reagent from another kit. We recommend that they be kept in the box in which they were supplied. If reagents are removed from the box please note on them the date shown on the bottom of the box so that specific lots of reagents can be traced.
7. Although the affinity-purified biotinylated secondary antibody and the normal serum provided in VECTASTAIN® Elite ABC Kits can be purchased individually, the Avidin DH and biotinylated horseradish peroxidase H are prepared especially for the VECTASTAIN® Elite ABC Kits and are matched reagents. Do not confuse these with Cat. Nos. A-2000 and B-2004. We recommend using only ABC reagents provided in the VECTASTAIN® Elite ABC kits.
8. For thicker sections, longer incubation times may be required for optimal staining.
9. To prevent sections from detaching from the glass, slides can be treated with VECTABOND™ Reagent (SP-1800), a non-protein tissue section adhesive. Do not use egg albumin coated slides. Traces of egg white avidin may affect staining quality.

Peroxidase Substrates

A variety of chromogens can be used to localize peroxidase in tissue or cell preparations. All Vector Laboratories' substrates are supplied in convenient, easy to use dropper bottles. Vector Laboratories offers conventional as well as proprietary substrates producing the colors listed.

Note: A chart of the Relative Sensitivity of Substrates in Immunohistochemistry and further description of substrate properties is available on our website: http://www.vectorlabs.com.

ImmPACT™ DAB EqV (Brown)	**SK-4103**	**400 ml**
ImmPACT™ DAB (Brown)	**SK-4105**	**120 ml**
ImmPACT™ AEC (Red)	**SK-4205**	**120 ml**
ImmPACT™ AMEC Red (Red)	**SK-4285**	**120 ml**
ImmPACT™ VIP (Purple)	**SK-4605**	**120 ml**
ImmPACT™ SG (Blue-Gray)	**SK-4705**	**120 ml**
ImmPACT™ NovaRED™ (Red)	**SK-4805**	**120 ml**
DAB (Brown or Gray-Black)	**SK-4100**	**1 kit**
AEC (Red)	**SK-4200**	**1 kit**
Vector® VIP (Purple)	**SK-4600**	**1 kit**
Vector® SG (Blue-Gray)	**SK-4700**	**1 kit**
Vector® NovaRED™ (Red)	**SK-4800**	**1 kit**
TMB (Blue)	**SK-4400**	**1 kit**

*AEC, ImmPACT™ AEC and ImmPACT™ AMEC Red must be mounted in aqueous mounting media. All other substrates may be dehydrated, cleared, and permanently mounted.

Kits contain sufficient reagent to prepare approximately 300 ml of working solution.

These substrates can be used as single labels or to introduce multiple colors in a tissue section.

VECTASTAIN® Elite ABC Kits

VECTASTAIN® Elite ABC Kit (Standard)	1 Kit	PK-6100
This Standard Kit consists of only the ABC Elitereagents.		
VECTASTAIN® Elite ABC Kit (Goat IgG)	1 Kit	PK-6105
VECTASTAIN® Elite ABC Kit (Human IgG)	1 Kit	PK-6103
VECTASTAIN® Elite ABC Kit (Mouse IgG)*	1 Kit	PK-6102
VECTASTAIN® Elite ABC Kit (Rabbit IgG)	1 Kit	PK-6101
VECTASTAIN® Elite ABC Kit (Rat IgG)	1 Kit	PK-6104
VECTASTAIN® Elite ABC Kit (Sheep IgG)	1 Kit	PK-6106
VECTASTAIN® Elite ABC Kit (Universal)	1 Kit	PK-6200

The VECTASTAIN® Elite ABC Reagent and VECTASTAIN® Elite ABC Universal Kit are available in ready-to-use (R.T.U.), prediluted formats.

R.T.U. VECTASTAIN® Elite ABC Reagent	50 ml	PK-7100
R.T.U. VECTASTAIN® Elite ABC Kit (Universal)	50 ml	PK-7200

* For staining mouse primary antibodies on mouse tissue, use the Vector® M.O.M.™ (Mouse on Mouse) Peroxidase Kit (PK-2200).

Biotinylated Antibodies Available

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

Biotinylated Anti-Cat IgG (H + L) made in goat	**1.5 mg**	**BA-9000**
Biotinylated Anti-Chicken IgG (H + L) made in goat	**1.5 mg**	**BA-9010**
Biotinylated Anti-Goat IgG (H + L)		
made in rabbit [a,d]	**1.5 mg**	**BA-5000**
made in horse [a]	**1.5 mg**	**BA-9500**
Biotinylated Anti-Guinea Pig IgG (H + L) made in goat	**1.5 mg**	**BA-7000**
Biotinylated Anti-Hamster IgG (H + L) made in goat	**1.5 mg**	**BA-9100**
Biotinylated Anti-Horse IgG (H + L) made in goat	**1.5 mg**	**BA-8000**
Biotinylated Anti-Human IgG (H + L) ◊ made in goat [d]	**1.5 mg**	**BA-3000**
Biotinylated Anti-Mouse IgG (H + L) ◊		
made in horse [d]	**1.5 mg**	**BA-2000**
made in goat	**1.5 mg**	**BA-9200**
Biotinylated Anti-Mouse IgG (H + L) (Rat Adsorbed) made in horse [b]	**0.5 mg**	**BA-2001**
Biotinylated Anti-Rabbit IgG (H + L)		
made in goat [d]	**1.5 mg**	**BA-1000**
made in horse	**1.5 mg**	**BA-1100**
Biotinylated Anti-Rat IgG (H + L)		
made in rabbit [d]	**1.5 mg**	**BA-4000**
made in goat	**1.5 mg**	**BA-9400**
Biotinylated Anti-Rat IgG (H + L)		
(Mouse Adsorbed) made in rabbit [c]	**0.5 mg**	**BA-4001**
(Mouse Adsorbed) made in goat [c]	**0.5 mg**	**BA-9401**
Biotinylated Anti-Sheep IgG (H + L) made in rabbit [a,d]	**1.5 mg**	**BA-6000**
Biotinylated Anti-Swine IgG (H + L) made in goat	**1.5 mg**	**BA-9020**
Biotinylated "Universal" Anti-Mouse/Rabbit IgG (H + L) made in horse [d,e]	**2.1 mg**	**BA-1400**
Biotinylated "Universal" Pan-Specific Anti-Mouse/Rabbit/Goat IgG (H + L) made in horse [f,g]	**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-1400) should be diluted 1:50 for use.
f - Universal Pan-Specific Anti-Mouse/Rabbit/Goat IgG (BA-1300) should be diluted 1:20.
g - Antibody used in the VECTASTAIN® Universal Quick Kits.

◊ Chain-specific antibodies are also available.

Related Reagents

Antigen Unmasking Solution (dilutes to 25 liters)		
Citrate-based	**250 ml**	**H-3300**
High pH	**250 ml**	**H-3301**
Avidin/Biotin Blocking Kit	**1 Kit**	**SP-2001**
BLOXALL™ Blocking Solution	**100 ml**	**SP-6000**
Bovine Serum Albumin (IHC grade)	**500 mg**	**SP-5050**
ImmEdge™ Hydrophobic Barrier Pen	**2-pen set**	**H-4000**
ImmPrint™ 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 QS	**100 ml**	**H-3404**
Vector® Methyl Green	**500 ml**	**H-3402**
Vector® Nuclear Fast Red	**500 ml**	**H-3403**
Heat-treated, ultrafiltered normal serum		
Normal Goat Serum	**20 ml**	**S-1000**
2.5% Normal Goat Serum	**50 ml**	**S-1012**
Normal Horse Serum	**20 ml**	**S-2000**
2.5% Normal Horse Serum	**50 ml**	**S-2012**
Normal Chicken Serum	**20 ml**	**S-3000**
Normal Swine Serum	**20 ml**	**S-4000**
Normal Rabbit Serum	**20 ml**	**S-5000**
Control Antibodies		
Rabbit IgG	**5 mg**	**I-1000**
Mouse IgG	**1 mg**	**I-2000**
Rat IgG	**1 mg**	**I-4000**
Goat IgG	**5 mg**	**I-5000**

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

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VECTASTAIN® Elite ABC Reagents and Kits are designed for laboratory use only.