



VECTASTAIN® *Elite* ABC KIT

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, very 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.

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
- 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® *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.

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 (Cat. No. 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 FOR PARAFFIN SECTIONS

1. Deparaffinize and hydrate tissue sections through xylenes or other clearing agents and graded alcohol series.
2. Rinse for 5 minutes in tap water. *
3. If quenching of endogenous peroxidase activity is required, incubate the sections in BLOXALL™ Blocking Solution for 10 minutes or in 0.3% H₂O₂ in either methanol or water for 30 minutes. Incubation times may be shortened by using higher concentrations of H₂O₂. If endogenous peroxidase activity does not present a problem, step 3 may be omitted.
4. Wash in buffer for 5 minutes.
5. Incubate sections for 20 minutes with diluted normal blocking serum which was prepared from the species in which the secondary antibody is made. (In cases where non-specific staining is not a problem, Steps 5 and 6 may be omitted).**
6. Blot excess serum from sections.
7. Incubate sections for 30 minutes with primary antibody diluted in buffer.
8. Wash slides for 5 minutes in buffer.
9. Incubate sections for 30 minutes with diluted biotinylated secondary antibody solution.
10. Wash slides for 5 minutes in buffer.
11. Incubate sections for 30 minutes with VECTASTAIN® *Elite* ABC Reagent.
12. Wash slides for 5 minutes in buffer.
13. Incubate sections in peroxidase substrate solution until desired stain intensity develops. For a list of peroxidase substrates see reverse.
14. Rinse sections in tap water.
15. Counterstain, clear and mount.

* If antigen unmasking is required, perform this procedure after step 2, using Antigen Unmasking Solution Citrate-based (Cat. No. H-3300) or High pH-based (Cat. No. H-3301).

** 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 (Cat. No. SP-2001) between steps 4 and 5.

STAINING PROCEDURE FOR FROZEN SECTIONS

This procedure is generally appropriate for frozen sections, cell smears or cytocentrifuge preparations.

1. Sections are air dried.
2. Immediately before staining, fix sections with acetone or the appropriate fixative for the antigen under study.
3. Transfer slides into buffer.
4. If quenching of endogenous peroxidase is required, use BLOXALL™ Blocking Solution for 10 minutes. Alternatively, use 0.3% H₂O₂ in 0.3% Normal Sera in PBS for 5 minutes; or 0.3% H₂O₂ in methanol for 30 minutes, or use other published methods (e.g. Andrew, S. M., Jasani, B., Histochem J. 1987, 19, 426-430).
5. Follow steps 4-15 of the procedure recommended for paraffin sections.

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, then add two drops of Reagent B. Mix and allow to stand for 5-30 minutes before use.

- 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.
- Wash gently with a stream of buffer from a wash bottle.
- If background staining is a problem, incubate sections for 5-10 minutes in 2%-10% normal serum in buffer.
- Incubate sections with primary antibody.†
- Wash as in step 3.
- Incubate sections for 10 minutes with diluted biotinylated secondary antibody.
- Wash as in step 3.
- Incubate sections for 5 minutes with VECTASTAIN® *Elite* ABC Reagent.
- Wash as in step 3.
- Incubate sections in peroxidase substrate solution until desired stain intensity develops.
- Wash as in step 3.
- 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 1 drop/2.5 ml. 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 (Cat. No. PK-8800), based on a preformed streptavidin/peroxidase complex, is also available to perform rapid immunohistochemical staining.

NOTES:

- 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.
- 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.
- The section should be well prepared. Fixation (generally, in buffered formalin not exceeding 4 percent formaldehyde) should be sufficient to maintain the integrity of the section throughout the staining procedure but not so harsh as to destroy the antigen under study. In some cases, use of Antigen Unmasking Solution (Cat. No. H-3300 or H-3301) and exposure to high temperatures can overcome loss of antigens due to fixation. During the staining procedure, do not allow the section to dry out. Use a humidified chamber for incubations.
- 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 0.1% immunohistochemical grade bovine serum albumin or dilute Blocking Serum.
- 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.

- 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.
- 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.
- Sections of neuronal tissue or thicker sections may require longer incubation times for optimal staining.
- To prevent sections from detaching from the glass, slides can be treated with VECTABOND™ Reagent (Cat. No. SP-1800), a non-protein tissue section adhesive. Do not use egg albumin coated slides. Traces of egg white avidin may affect staining quality.
- To minimize the risk of introducing contaminants, do not remove the drop dispensers from the stock solution bottles.

Peroxidase Substrates

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

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 are soluble in alcohol and clearing agents and must be mounted in aqueous mounting media. All other substrates are not soluble in alcohol or clearing agents. They 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.

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/catalog.aspx?catID=163>

VECTASTAIN® *Elite* ABC Kits

VECTASTAIN® <i>Elite</i> ABC Kit (Standard)	1 Kit	PK-6100
This Standard Kit consists of only the ABC Elitereagents.		
VECTASTAIN® <i>Elite</i> ABC Kit (Goat IgG)	1 Kit	PK-6105
VECTASTAIN® <i>Elite</i> ABC Kit (Human IgG)	1 Kit	PK-6103
VECTASTAIN® <i>Elite</i> ABC Kit (Mouse IgG)*	1 Kit	PK-6102
VECTASTAIN® <i>Elite</i> ABC Kit (Rabbit IgG)	1 Kit	PK-6101
VECTASTAIN® <i>Elite</i> ABC Kit (Rat IgG)	1 Kit	PK-6104
VECTASTAIN® <i>Elite</i> ABC Kit (Sheep IgG)	1 Kit	PK-6106
VECTASTAIN® <i>Elite</i> 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® <i>Elite</i> ABC Reagent	50 ml	PK-7100
R.T.U. VECTASTAIN® <i>Elite</i> 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 (Cat. No. 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 reconstituted with 2 ml water and 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
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

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.