



R.T.U. VECTASTAIN®

Elite®

ABC REAGENT

Catalog No. PK-7100

INSTRUCTIONS FOR IMMUNOHISTOCHEMICAL STAINING

Introduction

The VECTASTAIN® *Elite* ABC system is widely accepted as one of the most sensitive, economical and reliable immunoperoxidase systems available. The VECTASTAIN® ABC systems are extremely sensitive due to the form and number of active enzyme molecules associated with the preformed Avidin/Biotinylated enzyme Complex. This enhanced sensitivity 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 R.T.U. VECTASTAIN® *Elite* ABC Reagent is the same ABC reagent as that contained in other VECTASTAIN® *Elite* ABC Kits, but in a Ready-To-Use form.

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 and results in an increase in signal intensity with low background staining.

The R.T.U. 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

Due to the versatility of the avidin/biotin interaction, the R.T.U. VECTASTAIN® *Elite* ABC Reagent is modular and, along with our selection of secondary antibodies, can accommodate a wide array of primary antibody and tissue species.

COMPONENTS

Reagent supplied:

- 50 ml Ready-To-Use, stabilized *Elite* ABC Reagent.

The R.T.U. VECTASTAIN® *Elite* ABC Reagent will stain approximately 500 sections.

Storage:

R.T.U. VECTASTAIN® *Elite* ABC Reagent should be stored at 2-8 °C.

Reagents not supplied:

- Normal Blocking Serum
- Primary Antibody
- Biotinylated Secondary Antibody
- Buffer
- Hydrogen Peroxide
- Peroxidase Substrate

For convenience, the R.T.U. VECTASTAIN® *Elite* ABC Reagent is ready-to-use, in a convenient dropper bottle. (To remove the drop dispenser tip, press laterally with thumb until the tip snaps off.)

A number of different washing 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).

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 prediluted 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 prediluted biotinylated secondary antibody.

10. Wash for 5 minutes in buffer.

11. Incubate for 30 minutes with R.T.U. 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" (reverse).

14. Rinse 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

Rapid staining of tissue sections can be achieved using the VECTASTAIN® Universal Quick Kit (PK-8800) or its Ready-To-Use form (PK-7800) or by using any of the non-prediluted VECTASTAIN® *Elite* ABC Kits with the rapid staining protocol.

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. 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% H₂O₂ in tap water for 5 minutes or 0.3% H₂O₂ in either methanol or water for 30 minutes.

For frozen tissue or cell preparations, use 0.3% H₂O₂ in 0.3% normal serum in PBS for 5 minutes, or 0.3% H₂O₂ in methanol for 30 minutes or use other published methods. (eg. Andrew, S.M., Jasani, B., Histochem J. 1987, 19, 426-30).

- 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.
- Use only freshly prepared buffers. Bacterial contamination which can occur in buffers stored at room temperature may affect the quality of the staining.
- The affinity-purified biotinylated secondary antibody and the normal serum can be purchased individually (see reverse). The Avidin DH and biotinylated horseradish peroxidase H used to prepare the R.T.U. VECTASTAIN® *Elite* ABC Reagent are special. Do not confuse those in VECTASTAIN® ABC Kits with Cat. Nos. A-2000 and B-2004. We recommend using only ABC reagents provided in the VECTASTAIN® *Elite* ABC kits.
- For thicker sections, longer incubation times may be required for optimal staining.
- 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)	400 ml	SK-4103
ImmPACT™ DAB (Brown)	120 ml	SK-4105
ImmPACT™ AEC (Red)*	120 ml	SK-4205
ImmPACT™ AMEC Red (Red)*	120 ml	SK-4285
ImmPACT™ VIP (Purple)	120 ml	SK-4605
ImmPACT™ SG (Blue-Gray)	120 ml	SK-4705
ImmPACT™ NovaRED™ (Red)	120 ml	SK-4805
DAB (Brown or Gray-Black)	1 Kit	SK-4100
AEC (Red)*	1 Kit	SK-4200
Vector® VIP (Purple)	1 Kit	SK-4600
Vector® SG (Blue-Gray)	1 Kit	SK-4700
Vector® NovaRED™ (Red)	1 Kit	SK-4800
TMB (Blue)	1 Kit	SK-4400

*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 reagents 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

The R.T.U. VECTASTAIN® *Elite* ABC Reagent (PK-7100) and R.T.U. VECTASTAIN® "Universal" Kit (PK-7200) contain 50 ml of ready-to-use working solutions. Other VECTASTAIN® *Elite* ABC Kits provide 110 ml of working ABC solutions:

VECTASTAIN® <i>Elite</i> ABC Kit (Standard)	1 Kit	PK-6100
This Standard Kit consists of only the <i>Elite</i> ABC reagents.		
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
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 (PK-2200).

Biotinylated Antibodies

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 IgM μ chain specific, made in goat ^d	0.5 mg	BA-2020
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 (100x)		
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

VECTASTAIN® *Elite* ABC Reagents and Kits are designed to be used for laboratory use only. Detailed product listings, specifications and protocols are available on our website:

www.vectorlabs.com

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