VECTOR LABORATORIES

VECTASTAIN® ABC KIT

INSTRUCTIONS FOR IMMUNOHISTOCHEMICAL STAINING

INTRODUCTION

The VECTASTAIN® ABC Kit is a sensitive, low background, economical and reliable immunoperoxidase detection system.* The high sensitivity of the VECTASTAIN® ABC complex produced in this kit is due to the form and number of active enzyme molecules associated with the preformed Avidin/Biotinylated enzyme Complex (ABC). This 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). 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 - allow sensitive macromolecular complexes to be formed. The complexes remain stable for many hours after formation.

The VECTASTAIN® 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® 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® ABC Kit is modular and, along with our selection of secondary antibodies, can accommodate a wide array of primary antibody and tissue species.

* A further improvement in this original principle is the basis for a more sensitive version: the VECTASTAIN® *Elite* ABC Kit. Refer to our website for more information on the VECTASTAIN® *Elite* ABC Kits.

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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 orange-labeled small bottle – 2 ml
- Reagent B (Biotinylated Horseradish Peroxidase H) in brown-labeled small bottle – 2 ml

The VECTASTAIN® ABC Kit contains sufficient reagents to stain approximately 1000-2000 tissue sections.

NOTE: The VECTASTAIN® ABC Kit (Standard), Cat. No. PK-4000, contains only Reagent A and Reagent B.

Storage:

Stock VECTASTAIN® 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® 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 one (1) drop (50 µl) of stock (blue label) to 10 ml of buffer in mixing bottle (blue label).
- VECTASTAIN® ABC Reagent: add two (2) drops (100 µl) of REAGENT A (orange label) to 10 ml of buffer in the ABC Reagent mixing bottle. Then add two (2) (100 µl) drops of REAGENT B (brown label) to the same mixing bottle, mix immediately, and allow VECTASTAIN® ABC Reagent to stand for about 30 minutes before use.

For convenience, VECTASTAIN® 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 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. Care should be taken to replace the dispenser tip on the correct bottle to avoid cross contaminating reagents. All bottles have color coded labels to minimize inadvertent use of the wrong mixing bottle.

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® 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® 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.

NOTES:

1. VECTASTAIN® 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® 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. Incubation times may be shortened. In cases where the antigen concentration in the section is high, suggested incubation times with primary antibody, biotinylated secondary antibody, and VECTASTAIN® ABC Reagent may be reduced. Incubation times as short as five minutes have been reported to be sufficient in some cases when incubation temperatures are raised to 37 °C. If the antigen concentration is low, steps 7 and 9 may be lengthened to achieve maximal staining.

6. 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® 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.

7. 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.

8. Although the affinity-purified biotinylated secondary antibody and the normal serum provided in VECTASTAIN® ABC Kits can be purchased individually, the Avidin DH and biotinylated horseradish peroxidase H are prepared especially for the VECTASTAIN® 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® ABC kits.

9. For thicker sections, longer incubation times may be required for optimal staining.

10. 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.

11. 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 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® ABC Kits

VECTASTAIN® ABC Kit (Standard)	**1 Kit**	**PK-4000**
This Standard Kit consists of only the ABC reagents.		
VECTASTAIN® ABC Kit (Goat IgG)	**1 Kit**	**PK-4005**
VECTASTAIN® ABC Kit (Guinea Pig IgG)	**1 Kit**	**PK-4007**
VECTASTAIN® ABC Kit (Human IgG)	**1 Kit**	**PK-4003**
VECTASTAIN® ABC Kit (Mouse IgG)*	**1 Kit**	**PK-4002**
VECTASTAIN® ABC Kit (Mouse IgM)*	**1 Kit**	**PK-4010**
VECTASTAIN® ABC Kit (Rabbit IgG)	**1 Kit**	**PK-4001**
VECTASTAIN® ABC Kit (Rat IgG)	**1 Kit**	**PK-4004**
VECTASTAIN® ABC Kit (Sheep IgG)	**1 Kit**	**PK-4006**

* 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® 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® 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**

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VECTASTAIN® 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**