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## VECTASTAIN® ABC-AP KIT

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### INSTRUCTIONS FOR IMMUNOHISTOCHEMICAL STAINING

#### INTRODUCTION

The VECTASTAIN® ABC-AP Kit is a sensitive, low background, economical and reliable alkaline phosphatase detection system. The high sensitivity of the VECTASTAIN® ABC-AP complex produced in this kit is due to the form and number of active enzyme molecules associated with the preformed Avidin/Biotinylated enzyme Complex (ABCs). 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 alkaline phosphatase 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-AP 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-AP 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-AP 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 red-labeled small bottle - 2 ml
- Reagent B (Biotinylated Alkaline Phosphatase H) in red-labeled small bottle - 2 ml

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

**NOTE:** The VECTASTAIN® ABC-AP Kit (Standard), Cat. No. AK-5000, contains only Reagent A and Reagent B.

##### Storage:

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

##### Reagents not supplied:

- Primary Antibody
- Buffer
- Alkaline Phosphatase Substrate

#### PREPARATION OF VECTASTAIN® WORKING SOLUTIONS

A number of different buffers can be used in the VECTASTAIN® ABC-AP 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).
- Biotinylated Antibody: add one (1) drop (50 µl) of stock (blue label) to 10 ml of buffer in mixing bottle (blue label).
- VECTASTAIN® ABC-AP Reagent: add exactly two (2) drops (100 µl) of REAGENT A to 10 ml of buffer in the ABC Reagent mixing bottle. Then add exactly two (2) drops (100 µl) of REAGENT B to the same mixing bottle, mix immediately, and allow VECTASTAIN® ABC-AP Reagent to stand for about 30 minutes before use.

For convenience, VECTASTAIN® ABC-AP 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 top 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 top for refilling, merely press laterally with thumb until the top snaps off. Care should be taken to replace the dispenser top on the correct bottle to avoid cross contaminating reagents. All bottles have color-coded labels to minimize inadvertent use of the wrong mixing bottle. When dispensing drops, hold the bottle in an inverted vertical position and squeeze gently. To prevent evaporation, secure the white opaque caps on the bottles when they are not in use.

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

If more dilute reagents are used, first prepare the biotinylated antibody and VECTASTAIN® ABC-AP 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 BSA 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. Block endogenous enzyme activity. Endogenous alkaline phosphatase activity is less common in paraffin sections than in frozen tissue sections and is generally completely absent in sections treated with high temperature to unmask antigens. For effective and convenient blocking of all forms of alkaline phosphatase, incubate sections with one-step, ready-to-use BLOXALL™ Blocking Soluton (Cat. No. SP-6000) for 10 minutes. For alternative blocking protocols see note 2.
4. Wash in buffer for 5 minutes.
5. Incubate sections for 20 minutes with diluted normal blocking serum from the species in which the secondary antibody is made. (In cases where nonspecific 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 antiserum 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® ABC-AP Reagent.
12. Wash slides for 5 minutes in buffer.
13. Incubate sections for 20-30 minutes in alkaline phosphatase substrate solution. For a list of alkaline phosphatase substrates, see reverse.
14. Rinse sections in tap water.
15. Counterstain, clear and mount.

#### 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 directly into buffer.
4. Follow steps 3-15 of the procedure recommended for paraffin sections.

#### RAPID STAINING PROCEDURE

The sensitivity of the VECTASTAIN® ABC-AP Kit permits development of shortened alkaline phosphatase 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® ABC-AP protocol.

1. Prepare paraffin-embedded or frozen sections for staining as described previously.
2. Prepare VECTASTAIN® ABC-AP 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 ABC-AP Reagents, add two drops of Reagent A to 5.0 ml buffer, mix, then add two drops of Reagent B, mix. Allow to stand for 5-30 minutes before use.

- Block endogenous enzyme activity. Endogenous alkaline phosphatase activity is less common in paraffin sections than in frozen tissue sections and is generally completely absent in sections treated with high temperature to unmask antigens. For effective and convenient blocking of all forms of alkaline phosphatase, incubate sections with one-step, ready-to-use BLOXALL™ Blocking Solution (Cat. No. SP-6000) for 10 minutes. For alternative blocking protocols see note 2.
- Wash in buffer for 5 minutes.
- If background staining is a problem, incubate sections for 5-10 minutes in 2%-10% normal serum in buffer.
- Blot excess serum from sections.
- Incubate sections with primary antibody.\*
- Wash gently with a stream of buffer from a wash bottle.
- Incubate sections for 10 minutes with diluted biotinylated secondary antibody.
- Wash as in step 8.
- Incubate sections for 5 minutes with VECTASTAIN® ABC-AP Reagent.
- Wash as in step 8.
- Incubate sections in alkaline phosphatase substrate solution until desired stain intensity develops.
- Wash as in step 8.
- Counterstain, clear and mount.

\*The concentration, staining time, and incubation temperature is dependent upon the primary antibody used.

**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-AP Reagent as in the above protocol. Apply diluted VECTASTAIN® ABC-AP Kit reagents preheated to 37 °C. Incubate sections in each reagent for 2 minutes.

## NOTES:

- VECTASTAIN® ABC-AP 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](http://www.vectorlabs.com). Additional information on Enzyme Substrate Combinations, Counterstain/Substrate Compatibility, and Relative Substrate Sensitivity is also available on our website.
- Alternative methods for blocking endogenous alkaline phosphatase: If the endogenous activity is an isoenzyme other than the intestinal form it can be inhibited by adding Levamisole (Cat. No. SP-5000) to the substrate working solution. Intestinal alkaline phosphatase can be inhibited by following procedures described in the following reference: Bulman AS and Heyderman E; J. Clin. Pathol. 34, 1349-1351, 1981.
- 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. During the staining procedure, do not allow the section to dry out. Use a humidified chamber for incubations. In some cases, use of an Antigen Unmasking Solution, either Citrate-based (Cat. No. H-3300) or High pH (Cat. No. H-3301) and exposure to high temperatures can overcome loss of antigens due to fixation.

- 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 substrate solution be prepared with glass distilled water.
- Sections of neuronal tissue or sections which are thicker than normal 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.
- Although the affinity-purified biotinylated secondary antibody and the normal serum provided in VECTASTAIN® ABC-AP Kits can be purchased individually, the Avidin DH and biotinylated alkaline phosphatase H are prepared especially for the VECTASTAIN® ABC-AP Kits and are matched reagents. 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. Do not confuse these reagents with Cat. Nos. A-2000 and B-2005. We recommend using only ABC-AP reagents provided in the VECTASTAIN® ABC-AP Kits. The Avidin DH and biotinylated alkaline phosphatase H are available as the VECTASTAIN® ABC-AP Standard Kit (AK-5000).

## Alkaline Phosphatase Substrate Kits

A variety of chromogens can be used to localize alkaline phosphatase 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.

<b>Vector® Red</b>	<b>(Magenta)</b>	<b>1 Kit</b>	<b>SK-5100</b>
<b>Vector® Black</b>	<b>(Brown-Black)</b>	<b>1 Kit</b>	<b>SK-5200</b>
<b>Vector® Blue</b>	<b>(Blue)</b>	<b>1 Kit</b>	<b>SK-5300</b>
<b>BCIP/NBT</b>	<b>(Indigo)</b>	<b>1 Kit</b>	<b>SK-5400</b>

These alkaline phosphatase 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® ABC-AP Kits

Each kit contains sufficient reagents to prepare approximately 220 ml of each working solution. Generally 1000-2000 sections can be stained per kit.

<b>VECTASTAIN® ABC-AP Kit (Standard)</b>	<b>1 Kit</b>	<b>AK-5000</b>
This Standard Kit consists of only the ABC-AP reagent		
<b>VECTASTAIN® ABC-AP Kit (Goat IgG)</b>	<b>1 Kit</b>	<b>AK-5005</b>
<b>VECTASTAIN® ABC-AP Kit (Guinea Pig IgG)</b>	<b>1 Kit</b>	<b>AK-5007</b>
<b>VECTASTAIN® ABC-AP Kit (Human IgG)</b>	<b>1 Kit</b>	<b>AK-5003</b>
<b>VECTASTAIN® ABC-AP Kit (Mouse IgG)</b>	<b>1 Kit</b>	<b>AK-5002</b>
<b>VECTASTAIN® ABC-AP Kit (Mouse IgM)</b>	<b>1 Kit</b>	<b>AK-5010</b>
<b>VECTASTAIN® ABC-AP Kit (Rabbit IgG)</b>	<b>1 Kit</b>	<b>AK-5001</b>
<b>VECTASTAIN® ABC-AP Kit (Rat IgG)</b>	<b>1 Kit</b>	<b>AK-5004</b>
<b>VECTASTAIN® ABC-AP Kit (Sheep IgG)</b>	<b>1 Kit</b>	<b>AK-5006</b>
<b>VECTASTAIN® ABC-AP Kit (Universal)</b>	<b>1 Kit</b>	<b>AK-5200</b>

## Biotinylated Antibodies Available

The following biotinylated antibodies can be used in conjunction with the VECTASTAIN® ABC-AP Kit:

<b>Biotinylated Anti-Cat IgG (H + L)</b> made in goat	<b>1.5 mg</b>	<b>BA-9000</b>
<b>Biotinylated Anti-Chicken IgG (H + L)</b> made in goat	<b>1.5 mg</b>	<b>BA-9010</b>
<b>Biotinylated Anti-Goat IgG (H + L)</b> made in rabbit <i>a,d</i> made in horse <i>a</i>	<b>1.5 mg</b> <b>1.5 mg</b>	<b>BA-5000</b> <b>BA-9500</b>
<b>Biotinylated Anti-Guinea Pig IgG (H + L)</b> made in goat	<b>1.5 mg</b>	<b>BA-7000</b>
<b>Biotinylated Anti-Hamster IgG (H + L)</b> made in goat	<b>1.5 mg</b>	<b>BA-9100</b>

<b>Biotinylated Anti-Horse IgG (H + L)</b> made in goat	<b>1.5 mg</b>	<b>BA-8000</b>
<b>Biotinylated Anti-Human IgG (H + L) †</b> made in goat <i>d</i>	<b>1.5 mg</b>	<b>BA-3000</b>
<b>Biotinylated Anti-Mouse IgG (H + L) †</b> made in horse <i>d</i> made in goat	<b>1.5 mg</b> <b>1.5 mg</b>	<b>BA-2000</b> <b>BA-9200</b>
<b>Biotinylated Anti-Mouse IgG (H + L)</b> (Rat Adsorbed) made in horse <i>b</i>	<b>0.5 mg</b>	<b>BA-2001</b>
<b>Biotinylated Anti-Rabbit IgG (H + L)</b> made in goat <i>d</i> made in horse	<b>1.5 mg</b> <b>1.5 mg</b>	<b>BA-1000</b> <b>BA-1100</b>
<b>Biotinylated Anti-Rat IgG (H + L)</b> made in rabbit <i>d</i> made in goat	<b>1.5 mg</b> <b>1.5 mg</b>	<b>BA-4000</b> <b>BA-9400</b>
<b>Biotinylated Anti-Rat IgG (H + L)</b> (Mouse Adsorbed) made in rabbit <i>c</i> (Mouse Adsorbed) made in goat <i>c</i>	<b>0.5 mg</b> <b>0.5 mg</b>	<b>BA-4001</b> <b>BA-9401</b>
<b>Biotinylated Anti-Sheep IgG (H + L)</b> made in rabbit <i>a,d</i>	<b>1.5 mg</b>	<b>BA-6000</b>
<b>Biotinylated Anti-Swine IgG (H + L)</b> made in goat	<b>1.5 mg</b>	<b>BA-9020</b>
<b>Biotinylated "Universal" Anti-Mouse/Rabbit IgG (H + L)</b> made in horse <i>d,e</i>	<b>2.1 mg</b>	<b>BA-1400</b>
<b>Biotinylated "Universal" Pan-Specific Anti-Mouse/Rabbit/Goat IgG (H + L)</b> made in horse <i>f,g</i>	<b>2.2 ml</b>	<b>BA-1300</b>

*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 reconstituted with 2 ml water and diluted 1:50 for use. Do not use the Universal antibody to stain rodent or rabbit tissue because of cross reactivity with endogenous IgG.*

*f - Universal Pan-Specific Anti-Mouse/Rabbit/Goat IgG (BA-1300) should be diluted 1:20. Do not use this Pan-Specific antibody to stain rodent, rabbit, goat, sheep, or bovine tissue because of cross reactivity with endogenous IgG.*

*g - Antibody used in the VECTASTAIN® Universal Quick Kits.*

† Chain-specific antibodies are also available.

## Related Reagents

<b>Antigen Unmasking Solution (dilutes to 25 liters)</b>		
<b>Citrate-based</b>	<b>250 ml</b>	<b>H-3300</b>
<b>High pH</b>	<b>250 ml</b>	<b>H-3301</b>
<b>Avidin/Biotin Blocking Kit</b>	<b>1 Kit</b>	<b>SP-2001</b>
<b>BLOXALL™ Blocking Solution</b>	<b>100 ml</b>	<b>SP-6000</b>
<b>ImmEdge™ Hydrophobic Barrier Pen</b>	<b>2-pen set</b>	<b>H-4000</b>
<b>ImmPrint™ Histology Pen</b>	<b>5-pen set</b>	<b>H-6100</b>
<b>Vectabond™ Reagent (dilutes to 350 ml)</b>	<b>7 ml</b>	<b>SP-1800</b>
<b>VectaMount™ Mounting Medium</b>	<b>60 ml</b>	<b>H-5000</b>
<b>VectaMount™ AQ Mounting Medium</b>	<b>60 ml</b>	<b>H-5501</b>
<b>Vector® Hematoxylin</b>	<b>500 ml</b>	<b>H-3401</b>
<b>Vector® Hematoxylin QS</b>	<b>100 ml</b>	<b>H-3404</b>
<b>Vector® Methyl Green</b>	<b>500 ml</b>	<b>H-3402</b>
<b>Vector® Nuclear Fast Red</b>	<b>500 ml</b>	<b>H-3403</b>

<b>Heat-treated, ultrafiltered normal serum</b>		
<b>Normal Goat Serum</b>	<b>20 ml</b>	<b>S-1000</b>
<b>2.5% Normal Goat Serum</b>	<b>50 ml</b>	<b>S-1012</b>
<b>Normal Horse Serum</b>	<b>20 ml</b>	<b>S-2000</b>
<b>2.5% Normal Horse Serum</b>	<b>50 ml</b>	<b>S-2012</b>
<b>Normal Chicken Serum</b>	<b>20 ml</b>	<b>S-3000</b>
<b>Normal Swine Serum</b>	<b>20 ml</b>	<b>S-4000</b>
<b>Normal Rabbit Serum</b>	<b>20 ml</b>	<b>S-5000</b>

## Control Antibodies

<b>Goat IgG</b>	<b>5 mg</b>	<b>I-5000</b>
<b>Mouse IgG</b>	<b>1 mg</b>	<b>I-2000</b>
<b>Rabbit IgG</b>	<b>5 mg</b>	<b>I-1000</b>
<b>Rat IgG</b>	<b>1 mg</b>	<b>I-4000</b>

Detailed product listings, specifications and protocols are available on our website: [www.vectorlabs.com](http://www.vectorlabs.com)

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