GENERAL NOTES:

1. **The section should be well prepared. Fixation 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.**
2. **All reagents should be used at optimal concentration. For most applications the VECTASTAIN® ABC-GO procedure is substantially more sensitive than other glucose oxidase techniques. Consequently the primary antibody often can be used at higher dilution. 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 BSA or dilute Blocking Serum (2 drops of concentrated stock in 10 ml of buffer) included in the kit.**
3. **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-GO Reagent may be reduced. If the antigen concentration is low, steps 6, 8, 10 and 12 may be lengthened to achieve maximal staining. Elevation of the incubation temperature will shorten the development time in substrate.**
4. **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-GO Reagent and substrate solution be prepared in buffers made with glass distilled water.**
5. **Stock VECTASTAIN® ABC-GO Kit reagents should be stored under refrigeration. For best results, the VECTASTAIN® ABC-GO Kit reagents should be used before the date shown on the bottom of the box. 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.**
6. **Although the affinity-purified biotinylated secondary antibody and the normal serum provided in the VECTASTAIN® ABC-GO Kits can be purchased individually, the Avidin DH and biotinylated glucose oxidase H are prepared especially for the VECTASTAIN® ABC-GO Kits and are matched reagents. Do not confuse these with Cat. Nos. A-2000 and B-2006. We recommend using only ABC-GO reagents provided in the VECTASTAIN® ABC-GO Kits. Biotinylated secondary antibody and normal serum can be purchased separately, and the Avidin DH and biotinylated glucose oxidase H are available as the VECTASTAIN® ABC-GO Standard Kit.**
7. **Sections of neuronal tissue or sections which are thicker than normal may require longer incubation times for optimal staining.**
8. **Specimens should not be embedded in paraffin heated higher than 60 °C. Too much heat can destroy antigens.**
9. **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.**
10. **After mounting, paraffin sections should be dried in a hot air oven at 50-56 °C. Some slide warmers contain "hot spots" that can overheat tissues.**
11. **Hand lotions can cause sections to detach from slides or may prevent adequate penetration of reagents. Avoid touching rinse baths with oily hands.**
12. **Paraffin tissue blocks should be stored in sealed containers in a cool location.**
13. **Complete deparaffinization is important. Clearing agents and alcohol solutions should be changed regularly. All steps of the deparaffinization should be sufficiently long to completely remove the paraffin from the sections.**
14. **Although the most sensitive glucose oxidase-based system, the VECTASTAIN® ABC-GO is less sensitive than the peroxidase-based VECTASTAIN® ABC systems. A higher concentration of primary antibody is generally required for staining with the VECTASTAIN® ABC-GO than with the VECTASTAIN® ABC peroxidase systems.**

VECTASTAIN® ABC-GO Kits available:
Each kit contains sufficient reagents to prepare approximately 220 ml of each of the working solutions. Generally 1000-2000 sections can be stained per kit.

VECTASTAIN® ABC-GO Kit (Standard)	OK-3000
This kit consists of only the ABC-GO reagent	
VECTASTAIN® ABC-GO Kit (Goat IgG)	OK-3005
VECTASTAIN® ABC-GO Kit (Guinea Pig IgG)	OK-3007
VECTASTAIN® ABC-GO Kit (Human IgG)	OK-3003
VECTASTAIN® ABC-GO Kit (Human IgM)	OK-3009
VECTASTAIN® ABC-GO Kit (Mouse IgG)	OK-3002
VECTASTAIN® ABC-GO Kit (Mouse IgM)	OK-3010
VECTASTAIN® ABC-GO Kit (Rabbit IgG)	OK-3001
VECTASTAIN® ABC-GO Kit (Rat IgG)	OK-3004

In addition to the VECTASTAIN® ABC-GO Kits listed above. the following biotinylated antibodies can be obtained separately and used with the ABC-GO reagent from the VECTASTAIN® ABC-GO Standard Kit or from any other VECTASTAIN® ABC-GO Kit:

Biotinylated "Universal" Anti-Mouse/Rabbit IgG (H + L) made in horse	2.1 mg	BA-1400
Biotinylated "Universal" Pan-Specific Anti-Mouse/Rabbit/Goat IgG (H + L) made in horse	2.2 ml	BA-1300
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	1.5 mg	BA-5000
Biotinylated Anti-Goat IgG (H+L)** made in horse	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 IgA (α-chain specific) made in goat	0.5 mg	BA-3030
Biotinylated Anti-Human IgE (ε-chain specific) made in goat	0.5 mg	BA-3040
Biotinylated Anti-Human IgG (H+L)† made in goat	1.5 mg	BA-3000
Biotinylated Anti-Human IgG (γ-chain specific) made in goat	0.5 mg	BA-3080
Biotinylated Anti-Human IgM† (μ-chain specific) made in goat	0.5 mg	BA-3020
Biotinylated Anti-Human Kappa Chain (κ–chain specific) made in goat	0.5 mg	BA-3060
Biotinylated Anti-Human Lambda Chain (λ–chain specific) made in goat	0.5 mg	BA-3070
Biotinylated Anti-Mouse IgG (H+L)† made in horse	1.5 mg	BA-2000
Biotinylated Anti-Mouse IgG (H+L) made in goat	1.5 mg	BA-9200
Biotinylated Anti-Mouse IgG (H+L) (Rat Adsorbed) made in horse	0.5 mg	BA-2001
Biotinylated Anti-Mouse IgM† (μ-chain specific) made in goat	0.5 mg	BA-2020
Biotinylated Anti-Rabbit IgG (H+L)† made in goat	1.5 mg	BA-1000
Biotinylated Anti-Rat IgG (H+L)† made in rabbit	1.5 mg	BA-4000
Biotinylated Anti-Rat IgG (H+L) made in goat	1.5 mg	BA-9400
Biotinylated Anti-Rat IgG (H+L) (Mouse Adsorbed) made in rabbit	0.5 mg	BA-4001
Biotinylated Anti-Sheep IgG (H+L)† made in rabbit	1.5 mg	BA-6000
Biotinylated Anti-Sheep IgG (γ-chain specific) made in rabbit	0.5 mg	BA-6080
Biotinylated Anti-Swine IgG (H+L) made in goat	1.5 mg	BA-9020

***Use with Dog IgG primary antibodies **Use with Bovine IgG primary antibodies.**
†Antibodies included in VECTASTAIN® ABC-GO Kits.

Other related reagents also available are:

Antigen Unmasking Solution (dilutes to 25 liters)	250 ml	H-3300
Avidin/Biotin Blocking Kit	1 Kit	SP-2001
Bovine Serum Albumin (BSA)	500 mg	SP-5050
ImmEdge™ Pen	2-pen set	H-4000
Vectabond™ Reagent (dilutes to 350 ml)	7 ml	SP-1800
VectaMount™ Mounting Medium	60 ml	H-5000
Vector® Hematoxylin	500 ml	H-3401
Vector® Methyl Green	500 ml	H-3402
Vector® Nuclear Fast Red	500 ml	H-3403

Heat-treated, ultrafiltered normal serum from

Goat	20 ml	S-1000	Swine	20 ml	S-4000
Horse	20 ml	S-2000	Rabbit	20 ml	S-5000
Chicken	20 ml	S-3000			

Each kit provides sufficient stock reagents to prepare about 300 ml of substrate solution.

Glucose Oxidase Substrate Kit I (NBT)	Cat. No. SK-3100
Glucose Oxidase Substrate Kit II (TNBT)	Cat. No. SK-3200
Glucose Oxidase Substrate Kit III (INT)	Cat. No. SK-3300

A complete catalog is available on request.

VECTOR LABORATORIES

VECTASTAIN® ABC-GO KIT

INSTRUCTIONS FOR IMMUNOHISTOCHEMICAL STAINING

INTRODUCTION

Avidin is a 68,000 molecular weight glycoprotein with an extraordinarily high affinity (10^{15} M^{-1}) for the small molecular weight vitamin, biotin. Because this affinity is over one million times higher than that of antibody for most antigens, the binding of avidin to biotin (unlike antibody-antigen interactions) is essentially irreversible. In addition to this high affinity, the Biotin/Avidin System can be effectively exploited because avidin has four binding sites for biotin and most proteins (including antibodies and enzymes) can be conjugated with several molecules of biotin. These aspects provide the potential for macromolecular complexes to be formed between avidin and biotinylated enzymes.

An immunoperoxidase procedure based on these properties was devised for localizing a variety of histologically significant antigens and other markers. (Hsu SM, Raine L, Fanger H: *Am. J. Clin. Pathol.* **75, 734-738, 1981; Hsu SM, Raine L, Fanger H:** *J. Histochem. Cytochem.* **29, 577-580, 1981.) This technique employs unlabeled primary antibody, followed by biotinylated secondary antibody and then a preformed Avidin and Biotinylated horseradish peroxidase macromolecular Complex. This has been termed the ABC technique.**

VECTASTAIN® ABC Kits are offered with glucose oxidase (GO) as the enzyme marker. VECTASTAIN® ABC-GO Kits contain a special form of Avidin DH and biotinylated glucose oxidase H. Although the structure of the ABC-GO has not been determined, it is likely that the complex is similar to that of the avidin-biotinylated peroxidase ABC. It probably consists of many biotinylated glucose oxidase molecules crosslinked by avidin into a three dimensional array. The complex apparently has few exposed biotin residues but retains at least one biotin binding site. Formation of the complex is achieved by mixing Avidin DH and biotinylated glucose oxidase H in dilute solution and in defined amounts prior to use. The complex remains stable for several hours after formation and in some cases can be used for several days after preparation.

The VECTASTAIN® ABC-GO system has an advantage over peroxidase-based immunohistochemical techniques – namely, there is no endogenous glucose oxidase in mammalian tissues. This feature enables the VECTASTAIN® ABC-GO to be used without blocking endogenous peroxidase activity with H_2O_2/methanol, a procedure that can adversely affect cell morphology and destroy some antigens in tissues. However, the glucose oxidase ABC kit is the least sensitive of the VECTASTAIN® ABC systems.

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Email: vector@vectorlabs.com
Website: http://www.vectorlabs.com

The signal amplifications reported for the VECTASTAIN® ABC system are likely due to the number of active enzyme molecules associated with the complex and the rapid, irreversible interaction of the complex with biotinylated antibody. The low background staining obtainable with the VECTASTAIN® ABC Kits is probably due to the high dilutions of primary antisera and other reagents employed in the method, the quality of our affinity-purified biotinylated secondary antibodies, and the specially prepared Avidin DH and biotinylated enzyme.

VECTASTAIN® ABC-GO KIT INSTRUCTIONS

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) - 2 ml**
- **Reagent B (Biotinylated Glucose Oxidase H) - 2 ml**

NOTE: **The VECTASTAIN® ABC-GO Kit (Standard), Cat.No. OK-3000, contains only Reagent A and Reagent B.**

Reagents not supplied:

- **Primary Antibody**
- **Buffers**
- **Glucose Oxidase Substrate**

PREPARATION OF VECTASTAIN® WORKING SOLUTIONS

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 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 opaque white caps on the bottles when they are not in use.

When using dropper bottles to dispense reagents, apply a sufficient number of drops on the slide to cover the entire section. Slides should then be placed in a humidified chamber during the incubation period. Staining dishes or coplin jars may also be used in the staining procedure. To make up these working solutions, use the same drop/volume ratio as recommended in the instructions for preparation of dropper bottle reagents but increase the amounts as desired.

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

NOTE: **If more dilute reagents are used, first prepare the diluted biotinylated antibody and VECTASTAIN® ABC-GO reagent as described in the instructions. Subsequent dilutions should be made in a buffer containing 0.1%** immunohistochemical grade **bovine serum albumin (BSA). Only immunohistochemical grade BSA should be used, 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.**

*** one drop is approximately 50 µl.**

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 distilled water.**
3. **Wash in buffer for 20 minutes.**
4. **Incubate sections for 20 minutes with diluted normal serum (large yellow-labeled bottle, not included in OK-3000) from the species in which the secondary antibody is made. (In cases where nonspecific staining is not a problem, steps 4 and 5 may be deleted).**
5. **Blot excess serum from sections.**
6. **Incubate sections for 30 minutes with primary antiserum diluted in buffer.**
7. **Wash slides for 10 minutes in buffer.**
8. **Incubate sections for 30 minutes with diluted biotinylated antibody solution (large blue-labeled bottle, not included in OK-3000).**
9. **Wash slides for 10 minutes in buffer.**
10. **Incubate sections for 30-60 minutes with VECTASTAIN® ABC-GO Reagent.**
11. **Wash slides for 10 minutes in buffer.**
12. **Incubate sections for 15-30 minutes in glucose oxidase substrate solution in the dark.**
13. **Wash sections for 5 minutes in tap water.**
14. **Counterstain, clear and mount.**

STAINING PROCEDURE FOR FROZEN SECTIONS

The 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. **Follow steps 4-14 of the procedure recommended for paraffin sections.**

After completion of the staining procedure, dilute working solutions should be discarded, and the containers washed with distilled water and stored together with the stock reagents in the kit box.

SUBSTRATES

Glucose oxidase catalyzes the oxidation of ß-D-glucose, producing hydrogen peroxide and gluconic acid. By carrying out the reaction in the presence of a suitable electron carrier certain organic compounds can be reduced to give colored products. One such family of organic compounds is tetrazolium salts. A set of reagents for localizing glucose oxidase in tissues consists of glucose as the substrate, phenazine methosulfate (PMS) as an intermediate electron carrier, and a tetrazolium salt which upon reduction forms a highly colored, insoluble formazan. Commonly used tetrazolium salts are 2,2´,5,5´-tetra-p-nitrophenyl-3,3´-[3,3´-dimethoxy-4,4´-diphenylene] ditetrazolium chloride (called tetranitroblue tetrazolium or TNBT) which produces a black color; 2,2´-di-p-nitrophenyl-5,5´-diphenyl-3,3´-[3,3´-dimethoxy-4,4´-diphenylene]-ditetrazolium chloride (called nitroblue tetrazolium or NBT) which produces a purple-blue color; and 2-[4-iodophenyl]-3-[4-nitrophenyl]-5-phenyltetrazolium chloride (called iodonitrotetrazolium violet or INT) which produces a red color. 2-[2´-benzothiazolyl]-5-styryl-3-[4´-phthalhydrazidyl] tetrazolium chloride (BSPT) is a useful tetrazolium salt for electron microscopic applications as its formazan is osmiophilic.

Substrate kits are available for three of these tetrazolium systems. Each kit contains solutions of glucose, phenazine methosulfate and the appropriate tetrazolium salt in convenient dropper bottles. A substrate mixing bottle is included to prepare the working solution. Kits contain sufficient stock solutions to prepare approximately 300 ml of substrate.

Glucose Oxidase Substrate Kit I (NBT)	Cat. No. SK-3100
Glucose Oxidase Substrate Kit II (TNBT)	Cat. No. SK-3200
Glucose Oxidase Substrate Kit III (INT)	Cat. No. SK-3300

NOTES:

1. **PMS is very light sensitive and solutions containing it should be protected from light as much as possible. Little is known about the toxicity or carcinogenicity of the substrate components and, therefore, care should be taken in the handling and disposing of all the substrate reagents.**
2. **In some cases it may be necessary to filter the substrate solution prior to use.**
3. **The solubility and precipitate color of tetrazolium salts can vary somewhat from lot to lot.**
4. **Sections stained with NBT, TNBT or BSPT can be mounted in aqueous or non-aqueous mounting media. In the latter case, dehydration should be carried out rapidly. Sections stained with INT should be mounted in an aqueous mounting media.**
5. **In some cases, if the reaction of VECTASTAIN® ABC-GO with substrate is too rapid or too long, a non-specific precipitate can be deposited on the section. This and other properties of the reaction can be modified by altering the relative proportions of the substrate constituents.**

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

References discussing the use of glucose oxidase in immunohistochemistry and the chemistry involved in tetrazolium reactions are available upon request.