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.

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 pull the cap 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. * one drop is approximately 50 µl

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) (Cat. No., SP-5050). 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.
Glucose oxidase catalyzes the oxidation of D-glucose, producing hydrogen peroxide and glucono lactone. 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 2,5,5'-tetra-p-nitrophenyl-3,3'-[3,3'-dimethoxy-4,4'-diphenylene]diterrazolium chloride (called tetranitroblue tetrazolium or TNTB) which produces a black color; 2,2'-di-p-nitrophenyl-5,5'-diphenyl-3,3'-[3,3'-dimethoxy-4,4'-diphenylene]-diterrazolium chloride (called nitroblue tetrazolium or NBT) which produces a purple-blue color; and 2-[2-benzothiazolyl]-5-styryl-3-[4'-phthalaldehydrazyl] tetrazolium chloride (BSP) which is a useful tetrazolium salt for electron microscropic 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 (TNB) 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, 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, TNB or INT 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 medium.
5. In some cases, if the reaction of VECAStAIN® 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.

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 (Rabbit IgG) OK-3001

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-9900
Biotinylated Anti-Chicken IgG (H + L) made in goat 1.5 mg BA-9100
Biotinylated Anti-Goat IgG (H + L) ** made in goat 1.5 mg BA-5000
Biotinylated Anti-Goat IgG (H + L) made in horse 1.5 mg BA-9500
Biotinylated Goats IgG (H + L) made in horse 1.5 mg BA-9700
Biotinylated Anti-Hamster IgG (H + L) made in goat 1.5 mg BA-9100
Biotinylated Anti-Horse IgG (H + L) made in horse 1.5 mg BA-8000
Biotinylated Anti-Human IgA made in goat 0.5 mg BA-3030
Biotinylated Anti-Human IgA made in horse 0.5 mg BA-3040
Biotinylated Anti-Human IgM (r-specific) made in goat 0.5 mg BA-3060
Biotinylated Anti-Human IgM made in horse 0.5 mg BA-3080
Biotinylated Anti-Human IgG (H + L) made in horse 1.5 mg BA-9200
Biotinylated Anti-Human IgM (r-specific) made in goat 0.5 mg BA-3020
Biotinylated Anti-Human IgG (H + L) made in goat 1.5 mg BA-9200
Biotinylated Anti-Rat IgG (H + L) made in rabbit 1.5 mg BA-1000
Biotinylated Anti-Rat IgG (H + L) made in goat 1.5 mg BA-4000
Biotinylated Anti-Rat IgG (H + L) made in rabbit 1.5 mg BA-9400
Biotinylated Anti-Rat IgG (H + L) made in goat 1.5 mg BA-9400
Biotinylated Anti-Rat IgG (H + L) made in rabbit 1.5 mg BA-6000
Biotinylated Anti-Rat IgG (H + L) made in goat 1.5 mg BA-6000
Biotinylated Anti-Rat IgG (H + L) made in rabbit 1.5 mg BA-6000
Biotinylated Anti-Rat IgG (H + L) made in goat 1.5 mg BA-9200

*Use with Dog IgG primary antibodies **Use with Bovine IgG primary antibodies.

Other related reagents also available are:
Antigen Unmasking Solution (dilutes to 25 liters) Citrate-based 250 ml H-3300
High pH 250 ml H-3300
Avidin/Biotin Blocking Kit 1 Kit SP-2001
Bovine Serum Albumin (BSA) 500 mg SP-3050
ImmunEdge® Reactivity Reducing Pen 2 pen-set H-4000
ImmPrint® Histology Pen 5 pen-set H-6100
Vector® Mounting Medium 60 ml SP-1800
Vector® Mounting AQ Mounting Medium 60 ml H-5501
Vector® Hematoxylin 500 ml S-3401
Vector® Hematoxylin, US 100 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.

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Glucose Oxidase Substrate Kit II (TNB) Cat. No. SK-3200
Glucose Oxidase Substrate Kit III (INT) Cat. No. SK-3300

A complete catalog is available on request.