VECTOR LABORATORIES

R.T.U. VECTASTAIN®

(Anti-Mouse IgG/Rabbit IgG/Goat IgG)

UNIVERSAL *Quick* KIT

Catalog No. PK-7800

INSTRUCTIONS FOR RAPID IMMUNOHISTOCHEMICAL STAINING

The VECTASTAIN® Universal ***Quick*** Kit was developed using the most advanced biotin/streptavidin complex technology. The components in this kit have been designed to provide the high sensitivity of other biotin/streptavidin systems, but with an accelerated staining protocol utilizing a biotinylated secondary antibody that recognizes mouse, rat, rabbit, goat, sheep, and bovine primary antibodies. The VECTASTAIN® Universal ***Quick*** Kit allows a primary antibody from different species to be used at high dilution with a rapid staining protocol.

The R.T.U. VECTASTAIN® Universal ***Quick*** Kit is provided in a ready-to-use, prediluted form, containing 50 ml of working solutions sufficient to stain approximately 500 tissue sections.

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WORKING SOLUTIONS

The R.T.U. VECTASTAIN® Universal ***Quick*** Kit contains three reagents in ready-to-use form: (1) prediluted normal horse serum, (2) prediluted biotinylated secondary antibody, made in horse, which recognizes rabbit IgG, mouse IgG, goat IgG, as well as primary antibodies from less commonly used species such as rat, bovine and sheep, and (3) a preformed streptavidin/peroxidase complex solution. Each is supplied in convenient dropper bottles. When using dropper bottles to dispense reagents hold bottle in an inverted **vertical** position and squeeze gently. Apply enough reagent on the slide to cover the entire section. Slides should then be placed in a humidified chamber during the incubation period.

The R.T.U. VECTASTAIN® Universal ***Quick*** Kit contains the following reagents:

- 50 ml of prediluted blocking serum (normal horse serum, NHS)
- 50 ml of prediluted biotinylated pan-specific universal secondary antibody
- 50 ml of ready-to-use streptavidin/peroxidase preformed complex.

The working solutions in this kit are supplied in a ready-to-use form. No dilution is necessary. Reagents should be stored under refrigeration when not in use.

ENZYME SUBSTRATES

A variety of chromogens can be used to localize peroxidase in tissue sections. Vector Laboratories offers the traditional substrates DAB and AEC as well as several proprietary substrates, producing colors as listed below:

Diaminobenzidine (DAB), SK-4100, brown
DAB + Ni^{2+}, SK-4100, gray/black
Vector® VIP, SK-4600, purple
Vector® SG, SK-4700, blue-gray
Vector® NovaRED™, SK-4800, dark red
TMB, SK-4400, blue
3-amino-9-ethyl carbazole (AEC)*, SK-4200, red

* AEC is soluble in alcohol and clearing agents and must be mounted in aqueous mounting media. All other substrates are not soluble in alcohol or clearing agents. They may be dehydrated, cleared, and permanently mounted.

These substrates can be used as single labels or to introduce multiple colors in a tissue section.

STAINING PROCEDURE FOR PARAFFIN SECTIONS

1. Deparaffinize and hydrate tissue sections through xylenes or other clearing agents and graded alcohol series.
2. Rinse briefly in tap water then in PBS. *
3. Incubate sections for about 10 minutes in prediluted blocking serum.
4. Blot excess serum from sections.
5. Incubate sections in primary antibody diluted in buffer containing 1.5% blocking serum. **
6. Wash slides for 5 minutes in PBS.†
7. Incubate sections in prediluted biotinylated pan-specific universal secondary antibody for 10 minutes.
8. Wash sections for 5 minutes with PBS.†
9. Incubate sections in ready-to-use streptavidin/peroxidase complex reagent for 5 minutes.
10. Wash sections for 5 minutes with PBS.†
11. Incubate sections in peroxidase substrate solution until desired stain intensity develops. ‡
12. Rinse sections in tap water.
13. Counterstain, clear and mount.

* If endogenous peroxidase is present, incubate sections for 30 minutes in 0.3% H_2O_2 in methanol or 3% H_2O_2 in tap water for 5 minutes.

** The length of incubation times vary depending on the concentration of primary antibody. Generally, primary antibody concentrations should be such that optimal staining is achieved with incubation times of 15 minutes to 1 hour. Additional blocking serum can be purchased separately.

† In most cases, washes between reagent incubations can be shortened to brief rinses.

‡ See note 2 for approximate development times.

STAINING PROCEDURE FOR FROZEN SECTIONS

This procedure is generally appropriate for frozen sections, cell smears or cytocentrifuge preparations.

1. Use unfixed, acetone fixed or appropriate fixative for the antigen in question.
2. If quenching of endogenous peroxidase is required, use gentle H_2O_2 blocking to reduce the risk of antigen destruction or tissue loss: 0.3% H_2O_2 in 0.3% NHS 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). If necessary, H_2O_2 treatment may also be performed after the biotinylated secondary antibody step.
3. Follow steps 2-13 of the procedure recommended for paraffin sections.

NOTES:

1. Solutions containing sodium azide or other inhibitors of peroxidase activity should not be used in preparing the peroxidase substrate or added to the VECTASTAIN® Streptavidin/Peroxidase Complex Reagent. Do not add normal serum, non-fat dried milk, culture media or other potential sources of biotin to this reagent. This may result in reduced sensitivity.

2. Substrate development times may differ depending upon the level of antigen, the intensity of the stain that is required, or the substrate used. ImmPACT™ DAB and DAB generally should be developed for 2-10 minutes; ImmPACT™ VIP and Vector® VIP for 2-15 minutes; ImmPACT™ SG and Vector® SG for 2-10 minutes; ImmPACT™ NovaRED™ and Vector® NovaRED™ for 2-15 minutes; ImmPACT™ AEC and AEC for 10-30 minutes; TMB for 5-20 minutes. Some counterstains may not be compatible with certain peroxidase substrates because of solubility of the reaction products or lack of color contrast. A counterstain compatibility chart is available upon request. Refer to the instructions in the respective substrate kits for further details.

3. In the presence of nickel ions, the precipitate formed by DAB is gray/black rather than brown. This may enhance the sensitivity of the staining procedure and, because of the difference in color from DAB alone, has been used in double-labeling techniques. The DAB Substrate Kit (Cat. No. SK-4100) contains nickel chloride and allows two colors to be introduced into the section.

4. For some staining applications the reagents may be diluted beyond the concentrations supplied. Subsequent dilutions should be made in a buffer containing 0.1% immunohistochemical grade-bovine serum albumin. 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 elevated incubation temperatures to achieve maximum sensitivities.

5. The section should be well prepared. Fixation (generally, in buffered formalin not exceeding 4% 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. If staining is absent, unmasking of antigens may be required before the primary antibody can bind. Special Antigen Unmasking Solutions (Cat. No. H-3300 or H-3301) and a detailed protocol describing the method are available. During the staining procedure, do not allow the section to dry out. If necessary, use a humidified chamber for incubations.

6. To avoid adsorption of the primary 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 bovine serum albumin or dilute Blocking Serum.

7. 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. Deionized water (even with low conductivities) may contain inhibitors of peroxidase and can reduce sensitivity.

8. R.T.U. VECTASTAIN® Universal ***Quick*** Kit reagents should be stored under refrigeration. For best results, the R.T.U. VECTASTAIN® Universal ***Quick*** Kit reagents should be used before the date shown on the box. 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 box so that specific lots of reagents can be traced.

9. Sections of neuronal tissue or sections which are thicker than normal may require longer incubation times for optimal staining.

10. Specimens should not be embedded in paraffin heated higher than 60 °C. Too much heat can destroy antigens. 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. 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.

11. 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.

12. Hand lotions can cause sections to detach from slides or may prevent adequate penetration of reagents. Avoid touching rinse baths with oily hands.

VECTASTAIN® Universal ***Quick*** Kit Reagents and Kits are designed to be used for laboratory use only.

PEROXIDASE SUBSTRATE KITS

Peroxidase Substrates		
ImmPACT™ DAB (brown)	**120 ml**	**SK-4105**
ImmPACT™ AEC (red)	**120 ml**	**SK-4205**
ImmPACT™ VIP (purple)	**120 ml**	**SK-4605**
ImmPACT™ SG (blue/gray)	**120 ml**	**SK-4705**
ImmPACT™ NovaRED™ (red)	**120 ml**	**SK-4805**
DAB/Ni Substrate (brown or gray/black)	**1 Kit**	**SK-4100**
AEC Substrate (red)	**1 Kit**	**SK-4200**
TMB Substrate (blue)	**1 Kit**	**SK-4400**
Vector® VIP Substrate (purple)	**1 Kit**	**SK-4600**
Vector® SG Substrate (blue/gray)	**1 Kit**	**SK-4700**
Vector® NovaRED™ Substrate (red)	**1 Kit**	**SK-4800**

The kits provide sufficient stock reagents to prepare about 300 ml of substrate solution.

COUNTERSTAINS

• **Hematoxylin** H-3401 • 500 ml
Hematoxylin stains nuclei blue-violet with crisp nuclear detail. Our hematoxylin is especially designed for immunocytochemical applications and is based on Gill's formula — an alcohol-free solution containing no mercury. This formulation is also ideally suited for sections developed with alcohol-soluble enzyme reaction products, such as AEC.

• **Hematoxylin QS** H-3404 • 100 ml
Vector® Hematoxylin QS, a modification of Mayer's hematoxylin developed especially for immunocytochemistry, provides crisp blue-violet nuclear staining without obscuring antigen-specific chromogen deposition. Requiring no "blueing" step and less than 45 seconds to stain, Vector® Hematoxylin QS contains no mercury and is ready-to-use without filtration.

• **Methyl Green** H-3402 • 500 ml
Methyl Green can be used with a wide range of enzyme reaction products and is especially suited for multiple label applications. It is also ideal for black and white photography of immunohistochemically stained sections. Our improved formulation of this counterstain allows sections to be stained optimally using a simple, two-step protocol.

• **Nuclear Fast Red** H-3403 • 500 ml
Nuclear Fast Red stains nuclei pink to red. Tissue sections can be counterstained in a rapid, one-step protocol.

ADDITIONAL REAGENTS

• **Blocking Serum** S-2000 • 20 ml
The blocking serum contained in the VECTASTAIN® Universal ***Quick*** Kit is horse serum. Sera are obtained from healthy adult animals, heat treated at 56 °C for 2 hours, incubated at 4 °C to precipitate some of the cryoglobulins, ultracentrifuged and ultrafiltered through a 0.45µ filter. The horse serum is supplied undiluted with 0.08% sodium azide as a preservative.

• **VECTABOND™ Reagent** SP-1800 • 7 ml
VECTABOND™ Reagent is a novel tissue section adhesive that can significantly increase adherence of both frozen and paraffin embedded tissue sections to glass slides during standard immunohistochemical procedures, or under harsh conditions such as required for high temperature antigen unmasking techniques. This product does not coat slides with a glue or denatured protein, but chemically modifies the glass to form a highly adherent surface. Provided as a 50x concentrated stock sufficient for treating at least 500 slides.

• **Avidin/Biotin Blocking Kit** SP-2001 • 1 kit
• **Streptavidin/Biotin Blocking Kit** SP-2002 • 1 kit
These blocking kits consist of 18 ml of Avidin D or Streptavidin and 18 ml of biotin in convenient dropper bottles. These kits are designed for use in those cases when streptavidin, avidin, or biotinylated products bind nonspecifically to tissues or proteins.

• **Bovine Serum Albumin (BSA)** SP-5050 • 500 mg
Immunohistochemical Grade
This ultrapure grade of bovine serum albumin (BSA) can be used as a diluent or a blocking agent in numerous applications including ELISAs, blots and immunohistochemistry. This product is free of impurities present in some grades of BSA which can introduce artifacts or increase background staining in ELISAs, blot development, or immunohistochemical staining.

• **Control Antibodies**

Rabbit IgG	I-1000	• 5 mg
Mouse IgG	I-2000	• 1 mg
Goat IgG	I-5000	• 5 mg

These IgG preparations are intended for use as controls for primary antibodies made in rabbit or mouse.

• **Antigen Unmasking Solution**

Citrate-based	H-3300	• 250 ml
High pH	H-3301	• 250 ml

These formulas are highly effective at revealing antigens in formalin-fixed, paraffin-embedded tissue sections using a high temperature treatment procedure. The Antigen Unmasking Solution is supplied as an approximately 100x concentrated stock sufficient to prepare 25 liters of working solution. A detailed protocol describing optimal conditions for use is included.

• **Vectamount™ Mounting Medium** H-5000 • 60 ml
This toluene-free permanent mounting medium contains no hazardous chemicals, is odorless, dries clear with an ideal refractive index and shows no evidence of altering the color or intensity of any commonly used enzyme substrate with time.

• **VectaMount™ AQ Mounting Medium** H-5501 • 60 ml
This aqueous hard-setting mounting medium is designed for use with enzyme substrates, such as AEC, whose reaction products are soluble in alcohol or other organic solvents.

• **ImmEdge™ Hydrophobic Barrier Pen** H-4000 • 2-pen set
This hydrophobic barrier pen is lightly colored to be seen during and after application. The ImmEdge™ Pen keeps reagents localized to tissue sections, remains through all aqueous steps and is economical. Ideal for differentially staining two sections on the same slide.

• **ImmPrint™ Histology Pen** H-6100 • 5-pen set
This black permanent marking pen is resistant to most organic solvents encountered in histological applications and is designed to write on glass slides, tissue cassettes, and most hard surfaces.

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A comprehensive catalog of antibodies and other immunohistochemical products is available upon request or visit our website:

http://www.vectorlabs.com