



VECTASTAIN®
UNIVERSAL
Quick
KIT
Catalog No. PK-8800

INSTRUCTIONS FOR RAPID IMMUNOHISTOCHEMICAL STAINING

The VECTASTAIN® Universal *Quick* Kit is designed for rapid immunohistochemical staining of tissue sections. The kit relies on a proprietary preformed peroxidase-streptavidin complex to achieve outstanding sensitivity with short incubation times. This kit utilizes a biotinylated, universal, pan-specific secondary antibody that recognizes mouse, rat, rabbit, goat, sheep, and bovine primary antibodies.

The VECTASTAIN® Universal *Quick* Kit provides working solutions sufficient to stain approximately 250-500 tissue sections.

PREPARATION OF WORKING SOLUTIONS

The VECTASTAIN® Universal *Quick* Kit contains the following concentrated stock reagents:

- 6 ml blocking serum (normal horse serum, NHS)
- 2.2 ml biotinylated universal secondary antibody which recognizes rabbit IgG, mouse IgG, goat IgG, as well as primary antibodies from less commonly used species such as rat, bovine and sheep
- 1.2 ml of streptavidin/peroxidase preformed complex.

Each is supplied in convenient dropper bottles. When using dropper bottles to dispense reagents hold bottle in an inverted **vertical** position and squeeze gently. Working solutions can be prepared in glass test tubes. When applying working solutions, pipet enough reagent on the slide to cover the entire section. Slides should then be placed in a humidified chamber during the incubation period.

Working solutions are prepared in the following manner using PBS (10 mM phosphate, pH 7.5, 0.15M (0.9%) sodium chloride) as the diluent (1 drop \approx 50 μ l):

- Blocking solution: add 1 drop of blocking serum to 2 ml of PBS.
- Biotinylated Universal Secondary Antibody: add 4 drops of blocking serum plus 2 drops of biotinylated universal secondary antibody to 2 ml of PBS.
- Streptavidin/peroxidase preformed complex: add 1 drop of streptavidin/peroxidase complex stock solution to 2 ml of PBS.

The working solutions are ready to use immediately after dilution. Unused diluted working solution can be stored under refrigeration for 5 days without appreciable loss in sensitivity.

For added convenience, these reagents are now offered in stable, prediluted, ready-to-use solutions referred to as the R.T.U. VECTASTAIN® Universal *Quick* Kit (cat. no. PK-7800).

ENZYME SUBSTRATES

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

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

DAB (Diaminobenzidine), SK-4100, brown
DAB + Ni²⁺, SK-4100, gray-black
Vector® VIP, SK-4600, purple
Vector® SG, SK-4700, blue-gray
Vector® NovaRED™, SK-4800, red
TMB, SK-4400, blue
AEC (3-amino-9-ethyl carbazole)*, SK-4200, red
Vector Laboratories also offers a line of peroxidase substrates with increased sensitivity.

ImmPACT™ DAB EqV, SK-4103, brown
ImmPACT™ DAB, SK-4105, brown
ImmPACT™ VIP, SK-4605, purple
ImmPACT™ SG, SK-4705, blue-gray
ImmPACT™ NovaRED™, SK-4805, red
ImmPACT™ AEC*, SK-4205, red
ImmPACT™ AMEC Red*, SK-4285, red

* AEC, ImmPACT™ AEC and ImmPACT™ AMEC Red are 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.

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. If quenching of endogenous peroxidase activity is required, incubate the sections in **BLOXALL™** Blocking Solution (SP-6000) for 10 minutes or in 0.3% H₂O₂ in methanol or water for 30 minutes. Incubation times may be shortened by using higher concentrations of H₂O₂. If endogenous peroxidase activity does not present a problem, step 3 may be omitted.
4. Incubate sections for about 10 minutes in working solution of blocking serum.
5. Blot excess serum from sections.
6. Incubate sections in primary antibody diluted in buffer containing 1.5% blocking serum. *
7. Wash slides for 5 minutes in PBS.†
8. Incubate sections in biotinylated universal secondary antibody working solution for 10 minutes.
9. Wash sections for 5 minutes with PBS.†
10. Incubate sections in streptavidin/peroxidase complex working solution for 5 minutes.

11. Wash sections for 5 minutes with PBS.†
12. Incubate sections in peroxidase substrate solution until desired stain intensity develops.
13. Rinse sections in tap water.
14. Counterstain, clear and mount.

* 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.

STAINING PROCEDURE FOR FROZEN SECTIONS

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

1. Use fixation method appropriate for the antigen in question.
2. If quenching of endogenous peroxidase is required, use **BLOXALL™** Blocking Solution (SP-6000) for 10 minutes. Alternatively, use 0.3% H₂O₂ in 0.3% Normal Sera in PBS for 5 minutes; or 0.3% H₂O₂ in methanol for 30 minutes, or use other published methods (e.g. Andrew, S. M., Jasani, B., Histochem J. 1987, 19, 426-430).
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 diluting the peroxidase substrate or 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. For some staining applications the reagents may be diluted beyond their recommended concentrations. Subsequent dilutions should be made in a buffer containing 0.1% immunohistochemical grade Bovine Serum Albumin (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 elevated incubation temperatures to achieve maximum sensitivities.

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3. 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. Antigen Unmasking Solutions, Cat. No. H-3300 and H-3301, are available. During the staining procedure, do not allow the section to dry out. If necessary, use a humidified chamber for incubations.

4. 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.

5. 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® Streptavidin/Peroxidase Complex 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.

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

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

8. 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.

9. If smaller volumes of working solutions are desired, it is recommended that a drop from the stock solution be dispensed into a small, conical plastic tube. A suitable aliquot can then be withdrawn. To avoid the risk of introducing contaminants, do not remove the drop dispensers from the stock solution bottles.

PEROXIDASE SUBSTRATE KITS

Peroxidase Substrates

ImmPACT™ DAB EqV (brown)	400 ml	SK-4103
ImmPACT™ DAB (brown)	120 ml	SK-4105
ImmPACT™ AEC (red)	120 ml	SK-4205
ImmPACT™ AMEC Red	120 ml	SK-4285
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
Vector® 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 nuclear staining. This formula is ready-to-use without filtration, requires no separate "blueing" step, and stains in less than 45 seconds. Vector® Hematoxylin QS contains no mercury and provides excellent color contrast with most commonly used peroxidase and alkaline phosphatase substrates. This counterstain is suitable for use with non-aqueous and aqueous mountants.

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

BLOXALL™ Blocking Solution SP-6000 • 100 ml
BLOXALL™ inactivates endogenous peroxidase, pseudoperoxidase, and alkaline phosphatase in formalin-fixed, paraffin-embedded tissue sections, frozen tissue sections, and cell preparations. **BLOXALL™** Blocking Solution is provided ready-to-use in a convenient dropper bottle.

Blocking Serum

Normal Horse	S-2000	•	20 ml
2.5 % Normal Horse	S-2012	•	50 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 cryoglobulins, ultracentrifuged and ultrafiltered through a 0.45µ filter. Horse serum is also available as a 2.5% solution 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
Rat IgG	I-4000	•	1 mg
Goat IgG	I-5000	•	5 mg

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

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.

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

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

www.vectorlabs.com

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