The advanced avidin/biotin technology of the VECTASTAIN® ABC system results in an ABC complex that is smaller, uniform, and highly active. This allows more accessibility for binding to a biotinylated target and results in an increase in signal intensity with low background staining.

The R.T.U. VECTASTAIN® ABC 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

Due to the versatility of the avidin/biotin interaction, the R.T.U. VECTASTAIN® ABC Reagent is modular and, along with our selection of secondary antibodies, can accommodate a wide array of primary antibody and tissue species.

COMPONENTS

Reagent supplied:
- 50 ml Ready-To-Use, stabilized ABC Reagent.

The R.T.U. VECTASTAIN® ABC Reagent will stain approximately 500 sections.

Storage:
R.T.U. VECTASTAIN® ABC Reagent should be stored at 2-8 °C.

Reagents not supplied:
- Normal Blocking Serum
- Primary Antibody
- Biotinylated Secondary Antibody
- Buffer
- Hydrogen Peroxide
- Peroxidase Substrate

For convenience, the R.T.U. VECTASTAIN® ABC Reagent is ready-to-use, in a convenient dropper bottle. (To remove the drop dispenser tip, press laterally with thumb until the tip snaps off.)

A number of different washing buffers can be used in the VECTASTAIN® ABC system. One of the most common is 10 mM sodium phosphate, pH 7.5, 0.9% saline (PBS).

STAINING PROCEDURE

1. For paraffin sections, deparaffinize and hydrate through xylene's or other clearing agents and graded alcohol series.

2. If antigen unmasking is required, perform this procedure using a Vector® Antigen Unmasking Solution, Citrate-based (H-3300) or High pH-based (H-3301).

3. If quenching of endogenous peroxidase activity is required, incubate the slides in BLOXALL™ Blocking Solution (SP-6000) for 10 minutes. If endogenous peroxidase activity does not present a problem, this step may be omitted. For alternative quenching procedures please see Note 3.

4. Wash in buffer for 5 minutes.

5. Incubate for 20 minutes with prediluted normal blocking serum. (In cases where non-specific staining is not a problem, steps 5 and 6 can be omitted).*

6. Blot excess serum from sections.

7. Incubate for 30 minutes with primary antibody diluted in buffer (see Note 4).

8. Wash for 5 minutes in buffer.

9. Incubate for 30 minutes with prediluted biotinylated secondary antibody.

10. Wash for 5 minutes in buffer.


12. Wash for 5 minutes in buffer.

13. Incubate in peroxidase substrate solution until desired stain intensity develops. For a list of peroxidase substrates, see "Peroxidase Substrates" (reverse).

14. Rinse in tap water.

15. Counterstain, clear and mount.

* If unwanted staining occurs in the absence of biotinylated secondary antibody, endogenous protein-associated biotin may be present in the tissue. To eliminate this unwanted staining, use an Avidin/Biotin blocking step (SP-2001) between steps 4 and 5.

RAPID STAINING

Rapid staining of tissue sections can be achieved using the VECTASTAIN® Universal Quick Kit (PK-8800) or its Ready-To-Use form (PK-7800) or by using any of the non-prediluted VECTASTAIN® ABC Kits with the rapid staining protocol.

NOTES:

1. VECTASTAIN® ABC 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. Additional information on Enzyme Substrate Combinations, Counterstain/substrate Compatibility, and Relative Substrate Sensitivity is also available on our website.

2. Solutions containing sodium azide or other inhibitors of peroxidase activity should not be used in diluting the peroxidase substrate. Do not add normal serum, non-fat dried milk, culture media or other potential sources of biotin to the ABC reagent. This may result in reduced sensitivity.

3. Alternative peroxidase quenching procedures:
   - For formalin fixed cells and tissues, incubate in 3% H2O2 in tap water for 5 minutes or 0.3% H2O2 in either methanol or water for 30 minutes.
   - For frozen tissue or cell preparations, use 0.3% H2O2 in 0.3% normal serum in PBS for 5 minutes, or 0.3% H2O2 in methanol for 30 minutes or use other published methods. (eg. Andrew, S.M., Jasani, B., Histochem J. 1987, 19, 426-30).
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 diluted (2.5%) normal serum. Alternatively, 0.1% immunohistochemical grade bovine serum albumin (SP-5050) can be used. Other grades of BSA can contain undesired impurities.

5. Use only freshly prepared buffers. Bacterial contamination which can occur in buffers stored at room temperature may affect the quality of the staining.

6. The affinity-purified biotinylated secondary antibody and the normal serum can be purchased individually (see reverse). The Avidin DH and biotinylated horseradish peroxidase H used to prepare the R.T.U. VECTASTAIN® ABC Reagent are special. Do not confuse those in VECTASTAIN® ABC Kits with Cat. Nos. A-2000 and B-2004. We recommend using only ABC reagents provided in the VECTASTAIN® ABC kits.

7. For thicker sections, longer incubation times may be required for optimal staining.

8. To prevent sections from detaching from the glass, other substrates may be dehydrated, cleared, and permanently mounted. Kits contain sufficient reagents to prepare approximately 300 ml of working solution.

VECTASTAIN® ABC Kits

The R.T.U. VECTASTAIN® ABC Reagent (PK-7100) and the R.T.U. VECTASTAIN® Universal (PK-7200) contain 50 ml of ready-to-use working solutions. Other VECTASTAIN® ABC Kits provide 110 ml of working ABC solutions:

VECTASTAIN® ABC Kit (Standard) 1 Kit PK-6010
This Standard Kit consists of only the ABC reagents.

VECTASTAIN® ABC Kit (Goat IgG) 1 Kit PK-6015
VECTASTAIN® ABC Kit (Human IgG) 1 Kit PK-6013
VECTASTAIN® ABC Kit (Mouse IgG)* 1 Kit PK-6011
VECTASTAIN® ABC Kit (Rat IgG) 1 Kit PK-6010
VECTASTAIN® ABC Kit (Sheep IgG) 1 Kit PK-6005
VECTASTAIN® ABC Kit (Universal) 1 Kit PK-6010

R.T.U. VECTASTAIN® ABC Reagent 50 ml PK-7100
R.T.U. VECTASTAIN® ABC Kit (Universal) 50 ml PK-7200

* For staining mouse primary antibodies on mouse tissue, use the Vector® M.O.M.® (Mouse on Mouse) Peroxidase Kit (PK-2200).

Peroxidase Substrates

A variety of chromogens can be used to localize peroxidase in tissue or cell preparations. All Vector Laboratories’ substrates are supplied in convenient, ready-to-use dropper bottles. Vector Laboratories offers conventional as well as proprietary substrates producing the colors listed.

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.

VECTASTAIN® ABC Reagent 50 ml PK-7100

Biotinylated Antibodies

The following biotinylated antibodies can be used in conjunction with any VECTASTAIN® ABC Kit:

**Biotinylated Anti-Cat IgG (H + L)**
- 1.5 mg BA-9000 made in goat

**Biotinylated Anti-Chicken IgG (H + L)**
- 1.5 mg BA-9010 made in goat

**Biotinylated Anti-Goat IgG (H + L)**
- 1.5 mg BA-5000 made in rabbit
- 1.5 mg BA-9500 made in goat

**Biotinylated Anti-Guinea Pig IgG (H + L)**
- 1.5 mg BA-7000 made in goat

**Biotinylated Anti-Hamster IgG (H + L)**
- 1.5 mg BA-9100 made in mouse

**Biotinylated Anti-Horse IgG (H + L)**
- 1.5 mg BA-8000 made in goat

**Biotinylated Anti-Human IgG (H + L)**
- 1.5 mg BA-3000 made in goat

**Biotinylated Anti-Mouse IgG (H + L)**
- 1.5 mg BA-2000 made in mouse
- 1.5 mg BA-5200 made in goat

**Biotinylated Anti-Mouse IgM**
- 0.5 mg BA-2020 made in goat

**Biotinylated Anti-Mouse IgG (H + L) (Rat Adsorbed) made in horse**
- 0.5 mg BA-2001 made in goat

**Biotinylated Anti-Rabbit IgG (H + L)**
- 1.5 mg BA-1000 made in horse
- 1.5 mg BA-1100 made in goat

**Biotinylated Anti-Rat IgG (H + L)**
- 1.5 mg BA-4000 made in rabbit
- 1.5 mg BA-9400 made in goat

**Biotinylated Anti-Rat IgG (H + L) (Mouse Adsorbed) made in rabbit**
- 0.5 mg BA-4001 made in goat
- 0.5 mg BA-9401 made in goat

**Biotinylated Anti-Sheep IgG (H + L)**
- 1.5 mg BA-6000 made in goat

**Biotinylated Anti-Swine IgG (H + L)**
- 1.5 mg BA-9020 made in goat

**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

* For staining mouse primary antibodies on mouse tissue, use the Vector® M.O.M.® (Mouse on Mouse) Peroxidase Kit (PK-2200).

Related Reagents

Antigen Unmasking Solution (100x)
- Citrate-based 250 ml H-3300
- High pH 250 ml H-3301
- Avdin-Biotin Blocking Kit 1 Kit SP-2001
- BLOXX® Blocking Solution 100 ml SP-6000
- Bovine Serum Albumin (IHC grade) 500 mg SP-5050
- ImmEdge® Hydrophobic Barrier Pen 2-pen set H-4000
- ImmPrint® Histology Pen 5-pen set H-6100
- VECTABOND® Reagent (dilutes to 350 ml) 7 ml SP-1800
- VectaMount® Mounting Medium 60 ml H-5000
- VectaMount® AQ Mounting Medium 60 ml H-5501
- Vector® Hematoxylin 500 ml H-3401
- Vector® Hematoxylin QS 100 ml H-3402
- Vector® Methyl Green 500 ml H-3403
- Vector® Nuclear Fast Red 500 ml H-3403

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

Control Antibody

Rabbit IgG 5 mg I-1000
Mouse IgG 1 mg I-2000
Rat IgG 1 mg I-4000
Goat IgG 5 mg I-5000

VECTASTAIN® ABC Reagents and Kits are designed to be used for laboratory use only. Detailed product listings, specifications and protocols are available on our website:

www.vectorlabs.com

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