



## VECTASTAIN® ABC-AmP™

### Western Blotting Immunodetection Kit

#### Introduction

The VECTASTAIN® ABC-AmP™ Western Blotting Immunodetection Kit provides significant signal amplification using a special VECTASTAIN® ABC-AmP™ Reagent on nitrocellulose or polyvinylidene difluoride (PVDF) membranes. The VECTASTAIN® ABC-AmP™ Reagent is a pre-formed complex between streptavidin and biotinylated alkaline phosphatase. In addition to providing an amplified signal, the VECTASTAIN® ABC-AmP™ Reagent produces a very low background on these membranes. The substrate in these kits is either the chromogenic substrate for alkaline phosphatase, BCIP/NBT (5-Bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium), which produces a blue-purple precipitate, or the **DuoLuX™** Chemiluminescent/Fluorescent Substrate. The **DuoLuX™** Substrate provides very high sensitivity and has sustained light emission characteristics. Blots can be exposed to film several times over an 8 hour period for the optimization of band intensities or resolution. The reaction product of the **DuoLuX™** Substrate is also very fluorescent. These kits have been developed to produce maximum sensitivity with minimal background staining.

#### Kit Components

The components supplied in each kit provide sufficient reagents to develop approximately twenty 100 cm<sup>2</sup> blots.

- 10x Casein Solution (250 ml)
- Vector® Biotinylated Secondary Antibody (0.25 ml):
  - Anti-Mouse IgG (for mouse primary antibodies) or
  - Anti-Rabbit IgG (for rabbit primary antibodies)
- VECTASTAIN® ABC-AmP™ (0.5 ml Reagent A; 0.5 ml Reagent B)
- Substrate:
  - **DuoLuX™** Chemiluminescent/Fluorescent Substrate (100 ml) or
  - BCIP/NBT Chromogenic Substrate Kit (stock reagents for 200 ml)

#### Preparation of VECTASTAIN® ABC-AmP™ Working Solution

(For 100 cm<sup>2</sup> blot)

- 1x casein solution: Prepare 120 ml of 1x casein solution by adding 12 ml of 10x Casein Solution to 108 ml distilled water.
- Biotinylated secondary antibody solution: Add 10 µl of biotinylated anti-mouse IgG or anti-rabbit IgG to 10 ml of 1x casein solution for a final concentration of 1.5 µg/ml.
- VECTASTAIN® ABC-AmP™ reagent: Add 20 µl of Reagent A and 20 µl of Reagent B to 10 ml of 1x casein solution.

#### Detection Protocol

The volumes of the reagents in the procedure below are optimized for the development of a 100 cm<sup>2</sup> membrane. Volumes may be proportionally adjusted for blots of a different size.

1. Block the membrane in 10 ml of 1x casein solution for 5 minutes at room temperature with gentle agitation.

2. Incubate the membrane in an appropriate concentration of primary antibody diluted in PBS (10 mM Na<sub>2</sub>HPO<sub>4</sub>, pH 7.5, 150 mM NaCl) for 30 minutes at room temperature with gentle agitation (or for a time established to be optimal for the concentration of primary antibody used).
3. Wash the membrane in 10 ml of 1x casein solution 3 times for 4 minutes each at room temperature with gentle agitation.
4. Incubate the membrane for 30 minutes at room temperature with gentle agitation in 10 ml of the biotinylated secondary antibody solution appropriate for the species of primary antibody.
5. Wash as in Step 3.
6. Incubate the membrane in 10 ml of VECTASTAIN® ABC-AmP™ Reagent for 10 minutes at room temperature with gentle agitation.
7. Wash as in Step 3.
8. Incubate membrane in substrate solution as described in the appropriate section below. (It is recommended that the membrane be transferred to a different staining tray for the substrate development step.)

#### DuoLuX™ Substrate Development

(for Cat. No. AK-6601 or AK-6602)

#### Chemiluminescent signal acquisition:

- 8-a. Equilibrate membrane for 5 minutes in 0.1 M Tris buffer, pH 9.5.
- 8-b. Remove excess buffer by holding the membrane vertically and touching the edge of the membrane to absorbent paper.
- 8-c. Place membrane target-side-up on plastic wrap on a level surface.
- 8-d. Pipette 5 ml of **DuoLuX™** Substrate onto the membrane surface.

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8-e. Incubate for 5 minutes under subdued light or in the dark. Rinse the membrane in 0.1 M Tris buffer, pH 9.5, for a few seconds\* and remove excess buffer by holding the membrane vertically and touching the edge of the membrane to absorbent paper.

8-f. Place the membrane between two pieces of acetate, plastic wrap, or a clear sheet protector. Expose the membrane to x-ray film (such as KODAK BioMax™ film) for the appropriate time (see Note 5).

\* If necessary, background may be further reduced by washing the membrane in 0.1 M Tris buffer, pH 9.5, for 5 minutes at room temperature and removing excess buffer before exposure to film.

### Fluorescent signal acquisition:

After chemiluminescent signal acquisition, wash the membrane in 0.1 M Tris buffer, pH 9.5, for 5 minutes at room temperature and remove excess buffer. Place membrane target-side-up on a U.V. transilluminator (254-365 nm).

Fluorescent signal can be recorded using a CCD camera or traditional camera, each equipped with an ethidium bromide filter. Alternatively, the membrane can be epi-illuminated with U.V. light (254-365 nm). Pre-exposure of membrane to U.V. light for 2 minutes may enhance fluorescence. Exposure to U.V. light will abolish chemiluminescence. *If chemiluminescence detection is to be performed, it must be completed prior to fluorescence detection.*

### Chromogenic Substrate Development

(for Cat. No. AK-6401 or AK-6402)

8-a. Equilibrate membrane for 5 minutes in 0.1 M Tris buffer, pH 9.5.

8-b. Into 10 ml of 0.1 M Tris buffer, pH 9.5, add 4 drops of reagent from each of the three dropper bottles in the BCIP/NBT substrate kit.

8-c. Incubate membrane in the substrate solution at room temperature with gentle agitation until the appropriate density of colored bands develops. Incubation times may vary from 30 minutes up to several hours. Briefly rinse the membrane in PBS and air-dry. Store the blot protected from light.



### NOTES:

1. In rare cases some non-specific bands may develop. This can usually be eliminated by adding 180 mg NaCl to 10 ml of the VECTASTAIN® ABC-AmP™ Reagent. Overloading gels with too much protein may also produce spurious band staining. A range of concentrations of proteins can be applied in separate lanes to establish optimal results.
2. Some enzymes isolated from tissues may have covalently attached biotin as a co-factor. If high salt does not prevent the VECTASTAIN® ABC-AmP™ Reagent from binding to particular bands, use an Avidin/Biotin blocking step (Cat. No. SP-2001) between steps 1 and 2 in the protocol.
3. All kit reagents may be used immediately following dilution. For optimal results, it is recommended that all diluted reagents from the kit be used the same day that they are prepared. Stock VECTASTAIN® ABC-AmP™ Kit Reagents should be stored under refrigeration and kept in the box in which they are supplied.
4. Do not add any substances to the VECTASTAIN® ABC-AmP™ Reagent which may contain biotin or other inhibitors of Biotin/Avidin interactions. Such substances include serum, non-fat dry milk, culture media and some impure grades of bovine serum albumin.
5. Optimal exposure times can vary from 1 to 40 minutes. It is recommended that initial exposures be taken between 1 and 5 minutes. Band intensities or resolution can then be optimized by lengthening or shortening exposure times based on the initial results.

VECTASTAIN® ABC-AmP™ Western Blotting Immunodetection Kits available:

#### Chromogenic Detection Kits:

Anti-Mouse IgG	1 Kit	AK-6402
Anti-Rabbit IgG	1 Kit	AK-6401

#### Chemiluminescent Detection Kits:

Anti-Mouse IgG	1 Kit	AK-6602
Anti-Rabbit IgG	1 Kit	AK-6601

Other related reagents also available:

VECTASTAIN® ABC-AmP™ Reagent Standard Kit	1 Kit	AK-6000
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DuoLuX™ Chemiluminescent/Fluorescent Substrate for Alkaline Phosphatase	100 ml	SK-6605
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DuoLuX™ Chemiluminescent/Fluorescent Substrate for Peroxidase	200 ml	SK-6604
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BCIP/NBT Alkaline Phosphatase Substrate Kit	1 Kit	SK-5400
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10x Casein Solution	250 ml	SP-5020
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Biotinylated Anti-Rabbit IgG (H+L) made in goat*	1.5 mg	BA-1000
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Biotinylated Anti-Mouse IgG (H+L) made in horse*	1.5 mg	BA-2000
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Products available for western blot detection of tagged fusion proteins:

Biotinylated Anti-Maltose Binding Protein (MBP) made in goat	0.25 mg	BA-0701
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Biotinylated Anti-Green Fluorescent Protein (GFP) made in goat	0.25 mg	BA-0702
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Biotinylated Anti-C-MYC made in goat	0.25 mg	BA-0703
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Biotinylated Anti-HA made in goat	0.25 mg	BA-0704
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Biotinylated Anti-polyHistidine made in goat	0.25 mg	BA-0705
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\* Other species-specific biotinylated secondary antibodies are also available. See catalog or website for complete listing.

*This kit is designed for research use only.*

DuoLuX™ Chemiluminescent/Fluorescent Substrate contains a special formulation of Lumigen™ APS-5, Lumigen Inc. Southfield, MI. For reference, APS-5 is protected by the following patents:  
U.S. patent numbers: 6045727, 5922558, 6090571, 6296787, 7186568  
International patent numbers: 716233, 2213317, 179583, 819119, 3169383, 10-259144, 733086, 1019525, 743524, 1054933, 2002215610, 1322670