

VECTASTAIN® ABC-AmP Reagent (Standard)

Cat. No.: AK-6000

Storage: 2-8 °C

Instructions for western blot immunodetection.

DESCRIPTION

The VECTASTAIN ABC-AmP Western Blotting Immunodetection Kit provides significant signal amplification using a special VECTASTAIN ABC-AmP Reagent on nitrocellulose or PVDF membranes.

The VECTASTAIN ABC-AmP Reagent is a preformed complex between streptavidin and biotinylated alkaline phosphatase and can be used to detect any biotinylated antibody.

KIT COMPONENTS

Product	Volume
ABC-AmP Reagent A	0.5 ml
ABC-AmP Reagent B	0.5 ml

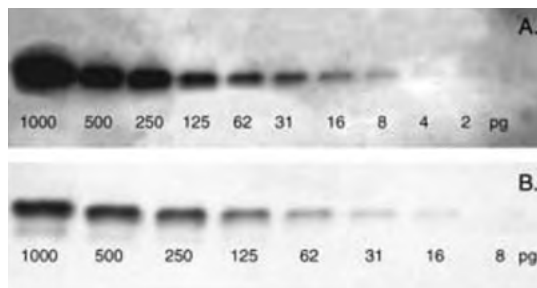
The VECTASTAIN ABC-AmP provides sufficient reagents to develop twenty 100 cm² blots.

STORAGE

- Store reagents in original bottles at 2-8 °C
- Do not freeze.

PREPARATION OF VECTASTAIN ABC-AmP WORKING SOLUTION (For 100 cm² blot)

VECTASTAIN ABC-AmP reagent: Add 20 µl of ABC-AmP Reagent A and 20 µl of ABC-AmP Reagent B to 10 ml of Casein Solution (SP-5020) or equivalent.



Western blot visualized by using the VECTASTAIN ABC-AmP kit. Serial dilutions (1:2) of maltose binding protein (MBP) were resolved by electrophoresis on a 12% PAGE reducing gel, transferred onto nitrocellulose membrane and detected with biotinylated anti-MBP made in goat followed by the VECTASTAIN ABC-AmP Reagent. Blots were developed with [A] DuoLuX® Substrate using chemiluminescence, or [B] BCIP/NBT Substrate.

DETECTION PROTOCOL

The volumes of the reagents in the procedure below are optimized for the development of a 100 cm² membrane. Volumes may be proportionally adjusted for blots of a different size.

1. Block the membrane in 10 ml of 1x casein solution or equivalent 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 Na₂HPO₄, 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 biotinylated secondary antibody appropriate for the species of primary antibody.
5. Wash in 10 ml of 1x casein solution 3 times for 4 minutes each.
6. Incubate the membrane in 10 ml of VECTASTAIN ABC-AmP Reagent for 10 minutes at room temperature with gentle agitation.
7. Wash in 10 ml of 1x casein solution 3 times for 4 minutes each.
8. Incubate membrane in an appropriate AP chemiluminescent such as DuoLuX Chemiluminescent Substrate (SK-6605) or precipitating substrate solution such as BCIP/NBT (SK-5400). (It is recommended that the membrane be transferred to a different staining tray for the substrate development step.)