

Maximize Sensitivity with the Amplified Alkaline Phosphatase Immun-Blot[®] Assay Kit

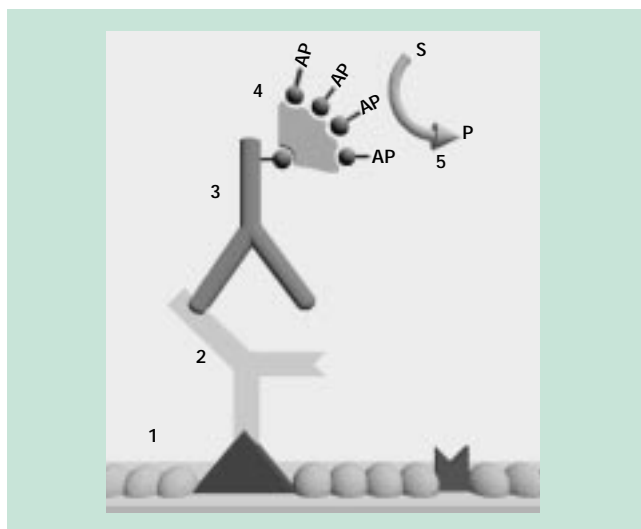


Fig. 1. Amplified detection of membrane bound antigens.
 1. Non-fat dry milk blocks unoccupied sites on the membrane.
 2. Primary antibody to a specific antigen is incubated with the membrane.
 3. Biotinylated antibody is added to bind to the primary antibody.
 4. Streptavidin-biotinylated-AP complex is incubated with the membrane.
 5. Color development reagent is then added to the blot. Amplified signal at the site of the complex is observed by the formation of a colored precipitate.

Highly Amplified Signals

The Amplified Alkaline Phosphatase Immun-Blot assay kit uses the high avidity of streptavidin-biotin binding to achieve improved sensitivity and reduce background in western blot immunoassays. With the new Amplified Alkaline Phosphatase Immun-Blot assay kit, 10 picograms of membrane bound protein can be easily detected. This is a 5-fold increase in sensitivity over results obtained with other non-isotopic western blot immunoassay systems.

Streptavidin-Biotin Complex Detection

This sensitivity is achieved by combining Bio-Rad's special production methods of biotinylating and affinity purifying our antibodies. Coupled with the use of streptavidin and biotinylated alkaline phosphatase, this system allows unsurpassed specificity of antigen binding while reducing non-specific background.

The rapid and essentially irreversible binding of biotin to streptavidin along with the ability to attach several biotin molecules to a single antibody molecule make this system ideal for any western blot.

Very Low Background

The Immun-Blot assay kit based on streptavidin offers many advantages, since streptavidin has properties that enable more specific binding than avidin. Avidin has an isoelectric point (pI) above 10, and, because it is glycosylated, the protein can stick, non-specifically, to DNA and cell membranes. In contrast, our purified streptavidin is not glycosylated and exhibits a pI between 5.5 and 6.5, the pH range in which most assays are performed. These properties make streptavidin ideal for immunoassay and DNA probe detection studies where non-specific binding to lectins, phospholipids, and nucleic acids can lead to high background.

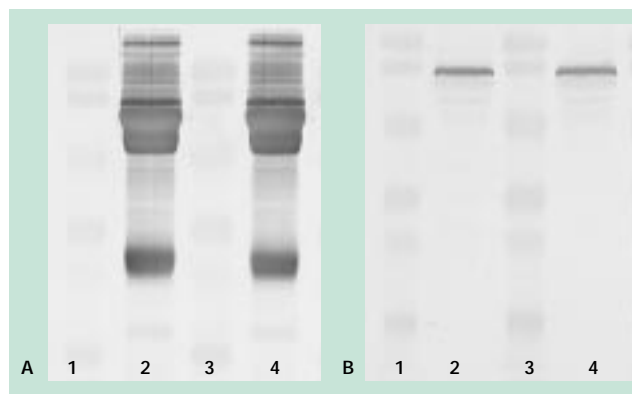


Fig. 2. The Amplified Alkaline Phosphatase Immun-Blot assay kit provides highly sensitive detection of antigens on Western blots. SDS-PAGE gels, 12%, were run in the Mini-PROTEAN II cell and transferred to nitrocellulose in the Mini Trans-Blot cell.

Total Protein Blot A: Lanes 1 and 3. Bio-Rad's Prestained LMW Standards. Lanes 2 and 4. Proteins labeled with Bio-Rad's NHS-Biotin. Labeled bands are detected with streptavidin-alkaline phosphatase complex and developed with AP color reagents.

Human Transferrin Blot B: Assayed using the Amplified Alkaline Phosphatase Immun-Blot assay protocol. Lanes 1 and 3. Bio-Rad's Prestained LMW Standards. Lanes 2 and 4. Human serum assayed with rabbit anti-human transferrin followed by the amplified alkaline phosphatase system.

Ease and Convenience in a Kit Form

Bio-Rad makes this new technology easy and convenient by providing a kit that contains our highest quality components in matched proportions and clear instructions to insure the success of every assay.

Each Amplified Alkaline Phosphatase Immun-Blot assay kit includes biotinylated Goat Anti-Rabbit IgG (H&L), human IgG adsorbed affinity purified antibody, purified streptavidin, biotinylated alkaline phosphatase, premixed Tris buffered saline, Tween 20, blotting grade blocker (non fat dry milk), and AP color reagents BCIP and NBT. The kit provides enough reagents to detect 300 strip assays or 30 mini blots. This is 10 times the number of assays available in other streptavidin-biotin kits. An assay is defined as the amount of reagent necessary for detection of antigen transferred to a 0.6-0.8 x 9.2 cm nitrocellulose strip when reacted in a total volume of 5.0 ml.

For more information on Bio-Rad's electrophoresis and blotting equipment, assay kits, and reagents for western blotting, contact your local technical representative.

Ordering Information

Catalog Number	Product Description
Immun-Blot Assay Kits	
170-6412	Amplified Alkaline Phosphatase Immun-Blot Assay Kit, Goat Anti-Rabbit IgG*
Premixed Enzyme Substrate Kit	
170-6432	Alkaline Phosphatase Conjugate Substrate Kit, contains premixed BCIP and NBT solutions and 10x color development buffer, prepares 1 L of color development solution
Individual Blotting Grade Reagents	
170-6401	Goat Anti-Rabbit IgG (H&L) Biotin Conjugate, 1 ml*
170-6403	Biotinylated Alkaline Phosphatase, 1 ml*
170-6404	Blocker, Non Fat Dry Milk, 300 g
170-6408	Streptavidin, 1 mg
170-6430	Premixed Tris Buffered Saline, 10x
170-6531	Tween 20, 100 ml

Ordering Information

Catalog Number	Product Description
170-6539	AP Color Development Reagent, BCIP (5 bromo-4-chloro-3-indolyl phosphate), 300 mg
170-6532	AP Color Development Reagent, NBT, (Nitro blue tetrazolium), 600 mg
Electrophoresis Standards	
161-0306	Biotinylated SDS-PAGE Standards, Low Range, 10,000-100,000 MW, 250 ml
161-0311	Biotinylated SDS-PAGE Standards, High Range, 45,000-210,000 MW, 250 ml
Blotting Membranes	
Nitrocellulose Membranes (0.45 micron)	
162-0115	Roll, 33 cm x 3 m, 1
162-0145	Sheets, 7 x 8.4 cm, 10
162-0116	Sheets, 15 x 15 cm, 10
Supported Nitrocellulose (0.45 micron)	
162-0094	Roll, 30 cm x 3 M
162-0090	Sheets, 7 x 8.4 cm, 10
162-0092	Sheets, 15 x 15 cm, 10
PVDF (0.2 micron)	
162-0184	Roll, 24 cm x 3.3 M
162-0186	Sheets, 7 x 8.4 cm, 10
162-0181	Sheets, 15 x 15 cm, 10
See catalog for more precut membrane sizes.	

* Dry ice shipment; additional freight charge will be added to final cost.

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