

DETECTION ON BLOTS

Immun-Star™ Chemiluminescent Kits for Detection of Western Blots

- Exceptional chemiluminescent detection on western blots
- Available with either alkaline phosphatase (AP) or horseradish peroxidase (HRP) conjugates
- Long-lasting, high-intensity signals
- Options for detection on X-ray film or charge-coupled device (CCD) imaging systems

Superior Sensitivity With Chemiluminescent Detection

Immun-Star chemiluminescent kits provide exceptional western blot detection. Bio-Rad offers several chemiluminescent detection kits based on HRP or AP secondary antibody conjugates with luminol or CDP-*Star* substrates. Kits include Immun-Star AP chemiluminescent kits, Immun-Star HRP chemiluminescent kits, and the Immun-Star WesternC™ chemiluminescent kit.

Chemiluminescent Western Blotting

Chemiluminescent detection is based on the interaction of a chemiluminescent substrate with an enzyme (HRP for the luminol substrate and AP for the CDP-*Star*) that has been conjugated to an antibody. Chemiluminescence is the light that is generated when the enzyme conjugate binds and interacts with the substrate. The light is captured on X-ray film in a standard darkroom cassette, and the film is developed to create a record of the blotting results. Alternatively, the blot can be exposed to an imaging screen on an instrument capable of detecting chemiluminescence, such as the Molecular Imager® VersaDoc™ MP and ChemiDoc™ XRS imaging systems, and digitally imaged with a computer.

Advantages of Immun-Star Kits

Long-Lasting Signal

Immun-Star reagents generate a strong signal that lasts for up to 24 hours, providing a great deal of flexibility in western blot detection — the long-lasting signal allows you to make multiple copies of a picture-perfect blot, or to expose the blot for different lengths of time to get the best signal possible. The signal can be regenerated even weeks after the initial activation by adding fresh substrate. Figures 1 and 2 show the long duration of the signal generated by Immun-Star products.

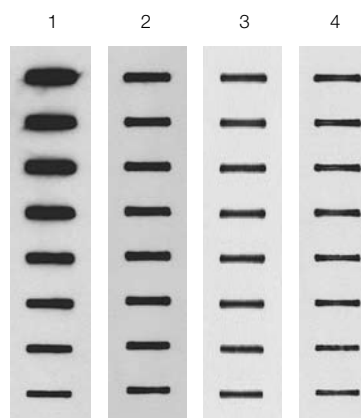


Fig. 1. Long-lasting Immun-Star AP signal, shown by a serial dilution of goat anti-rabbit (GAR) antibody on a slot blot. X-ray film exposures of 10 sec were made at the time of initial substrate activation (lane 1), 6 hr (lane 2), 12 hr (lane 3), and 24 hr (lane 4).



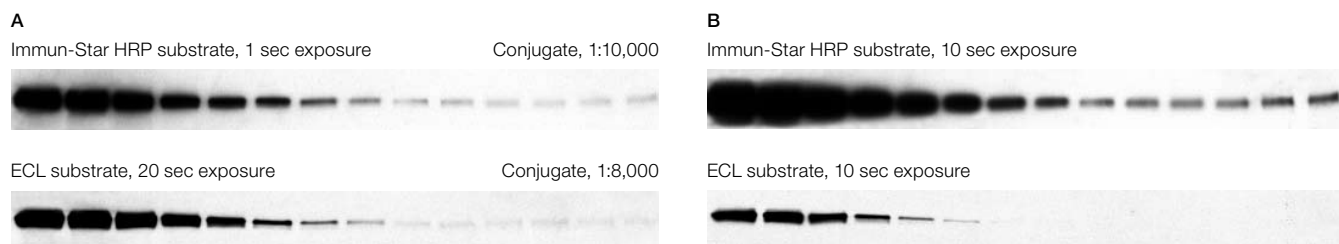


Fig. 2. Comparison of Immun-Star HRP kit and GE Healthcare ECL western blotting detection reagent. Two blots were prepared with serial dilutions of protein from 16 ng to 1.95 pg per well. Blots were incubated with Immun-Star HRP or ECL substrate and exposed to film. **A**, blot exposures showing the superior sensitivity of the Immun-Star HRP kit; **B**, blot exposures showing the extended signal duration of the Immun-Star HRP kit when the blots were exposed to film 2 hr after initial substrate activation.

High Sensitivity

Chemiluminescent western blot detection is a highly sensitive method for detecting proteins bound to blotting membranes. Picogram levels of protein can be detected with either the Immun-Star AP or the Immun-Star HRP kit, and the Immun-Star WesternC kit detects mid-femtogram amounts of protein. This sensitivity of protein detection is comparable to that of radioactive isotopic detection methods, but without the safety or disposal concerns associated with handling isotopes. Immun-Star kits can even surpass the performance of other chemiluminescent detection kits (Figure 2).

Flexible Membrane Compatibility

All Immun-Star chemiluminescent kits are compatible with PVDF and nitrocellulose membranes.

Fast Exposures

A significant advantage of chemiluminescent detection over alternative methods, especially radioactive methods, is the short exposure time required for maximum signal. Immun-Star kits offer very short exposure times for protein samples on nitrocellulose or PVDF membranes, typically only 30 seconds to a few minutes, even at low picogram levels.

Choice of Antibody Conjugates

Western blot detection involves many steps and reagents, and the substrate is not the only critical component to consider. Since nonspecific binding of an antibody increases background signals, the choice of the secondary antibody conjugate is also important.

The secondary antibodies used in the Immun-Star AP and HRP kits are provided as GAR or goat anti-mouse (GAM) conjugates and are specially formulated for western blot detection. The

conjugates are also affinity-purified and cross-adsorbed to eliminate cross-reacting antibody species. This ensures that specific binding of the secondary antibodies means strong signal production.

Convenient Kit Formats

Bio-Rad offers Immun-Star kits in a variety of configurations to allow you to select the substrate, antibodies, and reagents you need. For researchers new to the Immun-Star product line, the Immun-Star AP intro kits provide all the reagents needed for eight mini-blots in one kit. All kits provide an instruction manual, which includes reagent preparation methods, a detailed protocol, and an extensive troubleshooting guide. In addition, you can obtain a copy of Bio-Rad's protein blotting guide that provides information on protein transfer, detection methods, and troubleshooting advice by requesting bulletin 2895.

Membrane-Specific Information for the Immun-Star AP Kit

The membrane used for blotting determines how the Immun-Star AP substrate is used and whether an enhancer should be added. The chemical reaction is much faster and produces a stronger signal on a PVDF membrane than on nitrocellulose, so the substrate is used without an enhancer on most PVDF blots. If protein loads are low or if low-affinity antibodies are used, the enhancer may be included on PVDF blots. For nitrocellulose membranes, the enhancer should be added to the substrate prior to incubation of the blot. All Immun-Star AP kits feature ready-to-use substrate and enhancer. The substrate is in a 1x solution, and the enhancer is a 20x concentrate that is diluted directly in the substrate.

Immun-Star Chemiluminescent Kit Selection Guide

	Immun-Star HRP Kits	Immun-Star AP Kits	Immun-Star WesternC Kit
Sensitivity	1 pg	10 pg	Mid-femtogram
Signal duration	6–8 hr	24 hr	24 hr
Primary detection method	Film	Film	CCD imager
Recommended antibody dilution*	Primary: 1:1,000–1:6,000 Secondary: 1:15,000–1:30,000	Primary: 1:1,000–1:6,000 Secondary: 1:3,000	Primary: 1:10,000–1:50,000 Secondary: 1:50,000–1:250,000
Shelf life	4°C for 1 year	4°C for 1 year	Room temperature for 1 year
Recommended membrane	Nitrocellulose or PVDF	Nitrocellulose or PVDF	Nitrocellulose or PVDF

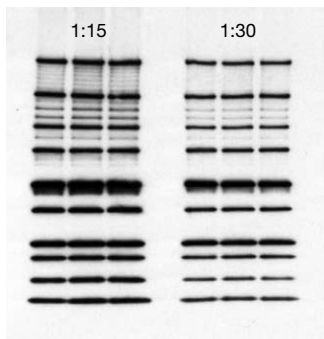
* 1 mg/ml starting concentration.

Compatibility With Standards

Immun-Star kits are ideal to use with Precision Plus Protein™ standards. Detecting Precision Plus Protein unstained standards and Precision Plus Protein™ WesternC™ standards directly on western blots is a straightforward procedure that utilizes conventional blotting methods and reagents.

Precision Protein™ StrepTactin-AP or -HRP conjugate can be incorporated into any blotting protocol without adding steps. Following electrophoresis, the protein gel samples (along with standards labeled with the *Strep*-tag sequence) are blotted onto a membrane. The blot is blocked, probed with the primary antibody of interest, washed, and then probed simultaneously with the secondary antibody and StrepTactin-AP or -HRP conjugate (both should contain the same enzyme conjugate). The standards, along with the antigen of interest, are visualized upon addition of Immun-Star HRP or AP substrate using X-ray film (Figure 3) or a Molecular Imager VersaDoc or ChemiDoc imaging system (Figure 4).

A. Immun-Star AP substrate



B. Immun-Star HRP substrate

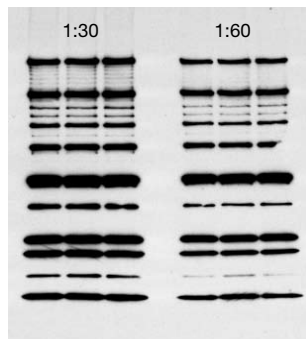


Fig. 3. Relative sensitivities of Immun-Star AP and HRP detection of Precision Plus Protein standards. **A**, 1:15 and 1:30 dilutions (235 and 119 ng of total protein per lane, respectively) of standards, detected with Immun-Star AP substrate; **B**, 1:30 and 1:60 dilutions (119 and 59 ng of total protein per lane, respectively) of standards, detected with Immun-Star HRP substrate. Both blots were exposed to X-ray film for 2 min.

CCD Imaging of Chemiluminescent Western Blots

CCD imagers offer the advantage of instant image capture and a larger dynamic range than film. The Immun-Star WesternC kit is designed to complement CCD imagers by offering strong and intense signals, mid-femtogram sensitivity (Figure 4), 24-hour signal duration for multiple exposures, and the option for image optimization using Quantity One® image analysis software. You can expect high-resolution images when using the Immun-Star WesternC chemiluminescent kit with CCD imagers. Qualitative and quantitative analyses of proteins on blots is more convenient than ever with this versatile kit.

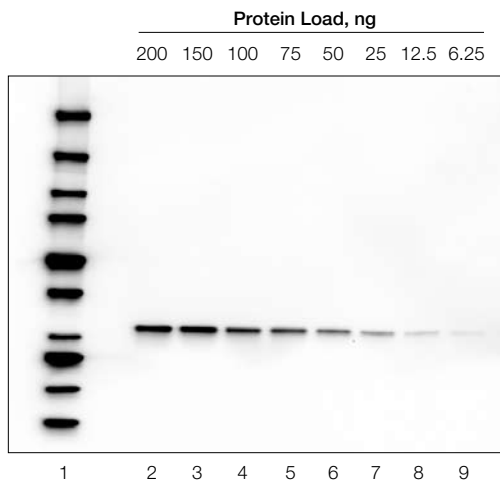


Fig. 4. Western blot detection using the Immun-Star WesternC kit.

Maximum sensitivity achievable with the Immun-Star WesternC kit is in the mid-femtogram range. A gel run with 5 μ l of Precision Plus Protein WesternC standards (lane 1) and a dilution series of *E. coli* lysate containing an overexpressed 27 kD protein (lanes 2–9) was transferred to a nitrocellulose membrane. The blot was probed with a primary antibody specific for the 27 kD protein and incubated with Precision Protein StrepTactin-HRP conjugate and a secondary antibody conjugated to HRP. After incubation in the Immun-Star WesternC detection solution for 5 min, the blot was imaged using the Molecular Imager ChemiDoc XRS system.

Ordering Information

Catalog # Description

Immun-Star WesternC Kit		Substrate	Enhancer	Antibody	TBS	Tween 20	Blocker
170-5070*	Substrate	•	•				

Immun-Star HRP Kits and Components		Substrate*	Antibody	TBS	Tween 20	Blocker
170-5040**	HRP Substrate, 500 ml	•				
170-5041*	HRP Substrate, 100 ml	•				
170-5042**	GAR-HRP Detection Reagents	•	•			
170-5043**	GAM-HRP Detection Reagents	•	•			
170-5044**	GAM-HRP Detection Kit	•	•	•	•	•
170-5045**	GAR-HRP Detection Kit	•	•	•	•	•
170-5046	GAR-HRP Conjugate		•			
170-5047	GAM-HRP Conjugate		•			

* Enough reagents are provided to cover 800 cm² of membrane (~15 mini blots).

** Enough reagents are provided to cover 4,000 cm² of membrane (~70 mini blots).

Immun-Star AP Kits and Components

170-5018*	AP Substrate	•				
170-5010*	GAM-AP Detection Kit	•	•	•		
170-5011*	GAR-AP Detection Kit	•	•	•		
170-5012*	AP Substrate Pack	•	•			
170-5013**	GAM-AP Intro Kit	•	•	•	•	•
170-5014**	GAR-AP Intro Kit	•	•	•	•	•
170-5015	Blotting Reagents Pack			•	•	•

* Enough reagents are provided to cover 2,500 cm² of membrane (~50 mini blots).

** Enough reagents are provided to cover 400 cm² of membrane (~8 mini blots).

Supporting Products

Catalog #	Description	Catalog #	Description	Catalog #	Description
Electrophoresis and Blotting Apparatus		Nitrocellulose/Filter Paper Sandwich (0.45 µm)*		Protein Standards	
165-6001	Criterion™ Cell	162-0234	Sheets, 8.5 x 13.5 cm, 20	161-0376	Precision Plus Protein WesternC Standards, 250 µl, 50 applications
170-4070	Criterion™ Blotter With Plate Electrodes	162-0235	Sheets, 8.5 x 13.5 cm, 50	161-0374	Precision Plus Protein Dual Color Standards, 500 µl, 50 applications
165-8000	Mini-PROTEAN® Tetra Cell	162-0214	Sheets, 7 x 8.5 cm, 20	161-0373	Precision Plus Protein All Blue Standards, 500 µl, 50 applications
170-3930	Mini Trans-Blot® Electrophoretic Transfer Cell	162-0215	Sheets, 7 x 8.5 cm, 50	161-0363	Precision Plus Protein Unstained Standards, 1 ml, 100 applications
Immun-Blot® PVDF Membrane		Nitrocellulose Membrane (0.2 µm)		161-0380	Precision Protein StrepTactin-HRP conjugate, 300 µl, 150 applications
162-0174	Sheets, 7 x 8.4 cm, 10	162-0112	Roll, 30 cm x 3.5 m, 1	161-0381	Precision Protein StrepTactin-HRP conjugate, 125 µl, 60 applications
162-0175	Sheets, 10 x 15 cm, 10	162-0146	Sheets, 7 x 8.4 cm, 10	161-0382	Precision Protein StrepTactin-AP conjugate, 300 µl, 150 applications
162-0176	Sheets, 20 x 20 cm, 10	162-0147	Sheets, 13.5 x 16.5 cm, 10		
162-0177	Roll, 26 cm x 3.3 m, 1	162-0150	Sheets, 20 x 20 cm, 5		
Immun-Blot PVDF/Filter Paper Sandwich*		Nitrocellulose Membrane (0.45 µm)			
162-0218	Sheets, 7 x 8.5 cm, 20	162-0113	Sheets, 20 x 20 cm, 5		
162-0219	Sheets, 7 x 8.5 cm, 50	162-0114	Sheets, 15 x 9.2 cm, 10		
162-0238	Sheets, 8.5 x 13.5 cm, 20	162-0115	Roll, 30 cm x 3.5 m, 1		
162-0239	Sheets, 8.5 x 13.5 cm, 50	162-0116	Sheets, 15 x 15 cm, 10		
Nitrocellulose/Filter Paper Sandwich (0.2 µm)*		162-0117	Sheets, 9 x 12 cm, 10		
162-0232	Sheets, 8.5 x 13.5 cm, 20	162-0145	Sheets, 7 x 8.4 cm, 10		
162-0233	Sheets, 8.5 x 13.5 cm, 50	162-0148	Sheets, 11.5 x 16 cm, 10		
162-0212	Sheets, 7 x 8.5 cm, 20				
162-0213	Sheets, 7 x 8.5 cm, 50				

* Membrane/filter paper sandwiches are 1 sheet of membrane and 2 sheets of thick filter paper precut to fit Criterion and mini gels.

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