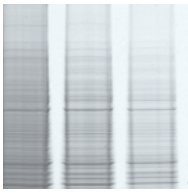
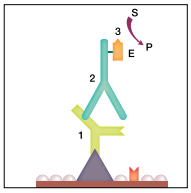




Western Blotting Detection Reagents

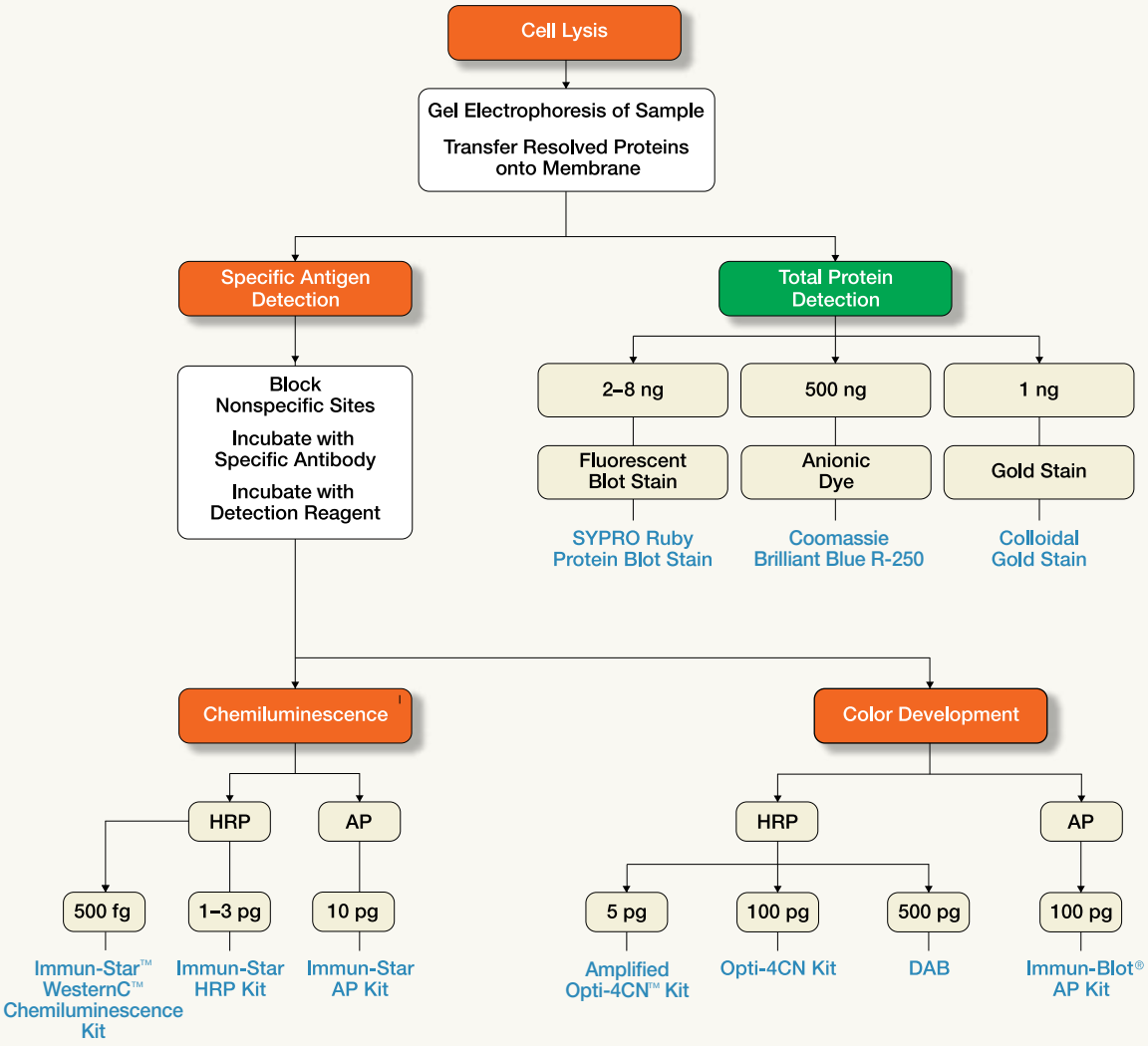
Maximize Western Blot Detection

BIO-RAD

Solutions for Any Blotting Application

Choose the Best Approach for Your Needs

When it comes to western blot detection, you can follow a number of different paths. Bio-Rad offers a complete line of reagents to meet virtually every possible need. This chart will help you choose an appropriate detection method and products for your application. Specific product information related to these methods can be found in later sections of this brochure. Most specific antigen detection methods are based on either horseradish peroxidase (HRP) or alkaline phosphatase (AP) secondary antibody conjugates, both of which are used to generate a visible signal.



Blot detection reagent selection guide.

Chemiluminescent Western Blot Detection

Superior Sensitivity

Chemiluminescent western blot detection is a highly sensitive alternative to isotopic detection. Instead of radioactively labeled antibodies, enzyme-conjugated antibodies are used to convert a substrate to one that produces a light signal. The signal can be captured on film or by dedicated imaging equipment.

Bio-Rad offers chemiluminescent detection based on luminol or CDP-*Star* substrates to generate fast, sensitive results on nitrocellulose or PVDF membrane blots.

Immun-Star HRP Kits With Luminol Substrate

If your secondary antibody is conjugated to HRP, choose the Immun-Star HRP kit for an excellent signal-to-noise ratio (Figure 1). Peroxidase-catalyzed oxidation of luminol produces the light signal. Blots can be stripped and reprobed multiple times.

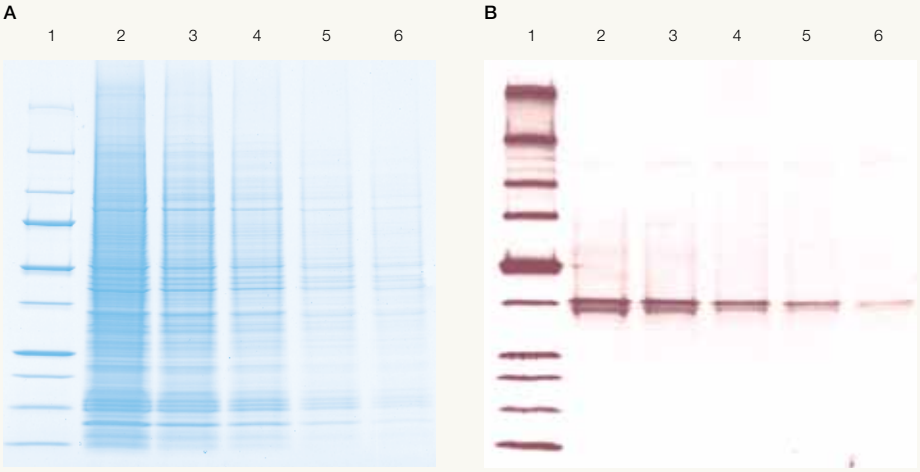


Fig. 1. Detection of CDK7 and Precision Plus Protein™ unstained standards using the Immun-Star HRP chemiluminescence detection kit. A, 10 µl of standards (lane 1) and a dilution series of a HeLa cell lysate (lanes 2–6) were electrophoresed on a 4–20% Criterion™ gel. The gel was stained with Bio-Safe™ Coomassie stain to visualize total protein; **B**, proteins from an identical gel, except with 0.5 µl of standards, were transferred to a nitrocellulose membrane. The optimal amount of standards to load on the blot was first determined using a dilution series. The blot was probed with an antibody specific for human CDK7 followed by an HRP-conjugated secondary antibody and StrepTactin-HRP conjugate. After a 2 min incubation in the Immun-Star HRP detection solution, the blot was exposed to film for 5 sec.

Detection Method	Substrate	Detection Sensitivity	Product Options	Advantages	Disadvantages
Chemiluminescent HRP	Luminol	1–3 pg	• Conjugates • HRP substrate • Immun-Blot kits	• Short (30 sec) exposure • Signal duration 6–8 hr • Compatible with PVDF and nitrocellulose • Working solution stable for 24 hr at room temperature	• Azide inhibits enzyme activity
Chemiluminescent AP	CDP-*Star*	10 pg	• Conjugates • AP substrate • Immun-Blot kits	• 30 sec to 5 min exposure • Signal continues for 24 hr after activation • Blot can be reactivated	• Detects endogenous phosphatase activity, which may lead to false positives

Immun-Star WesternC Chemiluminescence Kit

Charge-coupled device (CCD) imagers offer the advantages of instant image capture and a larger dynamic range than film-based systems. The Immun-Star WesternC chemiluminescence kit is designed to complement CCD imagers by offering strong and intense signals with a 24-hour signal duration for multiple exposures and for optimization of the images using Quantity One® software. Customers using the Immun-Star WesternC chemiluminescence kit for CCD imaging can expect improved quantitative results for each experiment compared with what can be obtained by film-based systems.

Comparison of Immun-Star Chemiluminescence Kits

	Immun-Star HRP Kits	Immun-Star AP Kits	Immun-Star WesternC Kit
Sensitivity	1–3 pg	10 pg	Mid-femtogram
Signal duration	6–8 hr	24 hr	24 hr
Primary detection method	Film	Film	CCD imager
Recommended antibody dilutions*	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.

Immun-Star AP Kits With CDP-*Star* Substrate

If your secondary antibody is conjugated to AP, choose Immun-Star AP for long-lasting signals that allow flexibility in obtaining data. An AP-catalyzed reaction of the chemiluminescence substrate CDP-*Star* produces the light signal (Figures 2 and 3). Blots can be reactivated, even weeks later, with the addition of fresh substrate.

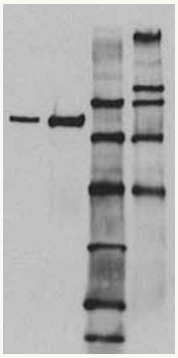


Fig. 2. Detection of transferrin using the Immun-Star AP chemiluminescence detection kit. Left to right, 1:2,000 and 1:200 dilutions of human transferrin, and low range and high range biotinylated standards; protein detected with Immun-Star AP substrate and enhancer on nitrocellulose. Film exposure time was 30 sec.

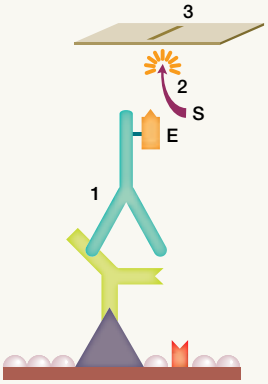


Fig. 3. Immun-Star chemiluminescent detection.
1. AP-conjugated secondary antibody binds to primary antibody.
2. AP (E) converts chemiluminescent substrate (S), which emits light.
3. Film or phosphor screen exposed by emitted light.

Ordering Information

Catalog #	Description	Substrate	Antibody	TBS	Tween 20	Blocker	Enhancer
Immun-Star HRP Kits and Components							
170-5040	**Immun-Star HRP Substrate**, 500 ml	•	–	–	–	–	–
170-5041	**Immun-Star HRP Substrate**, 100 ml	•	–	–	–	–	–
170-5043	**Goat Anti-Mouse (GAM)-HRP Detection Reagents**, 500 ml	•	•	–	–	–	–
170-5042	**Goat Anti-Rabbit (GAR)-HRP Detection Reagents**, 500 ml	•	•	–	–	–	–
170-5044	**Goat Anti-Mouse (GAM)-HRP Detection Kit**, 500 ml	•	•	•	•	•	–
170-5045	**Goat Anti-Rabbit (GAR)-HRP Detection Kit**, 500 ml	•	•	•	•	•	–
170-5047	**Goat Anti-Mouse (GAM)-HRP Conjugate**, 2 ml	–	•	–	–	–	–
170-5046	**Goat Anti-Rabbit (GAR)-HRP Conjugate**, 2 ml	–	•	–	–	–	–
Immun-Star AP Kits and Components*							
170-5010	**Goat Anti-Mouse (GAM)-AP Detection Kit**	•	•	–	–	–	•
170-5011	**Goat Anti-Rabbit (GAR)-AP Detection Kit**	•	•	–	–	–	•
170-5012	**AP Substrate Pack**	•	–	–	–	–	•
170-5018	**Immun-Star AP Substrate**	•	–	–	–	–	–
Immun-Star WesternC Chemiluminescence Kit							
170-5070	**Immun-Star WesternC Chemiluminescence Kit**, includes 50 ml luminol/enhancer, 50 ml stable peroxide buffer, enough for 1,000 cm^2 of membrane						

* All items cover 2,500 cm^2 of membrane. Combine the blotting reagents pack with a detection kit to form a complete blotting system. Enhancer is used on nitrocellulose blots, but in most cases not on PVDF blots.

Total Protein Stains | Discover the Pattern

Total protein staining of western blots provides a visual image of the electrophoretic pattern, which helps identify specific antigens in a complex protein mixture (Figure 4).

Methods for detecting proteins on membranes include staining with anionic dyes (such as Coomassie Blue and colloidal gold stains). Colloidal gold binds all proteins on a blot (Figure 5).

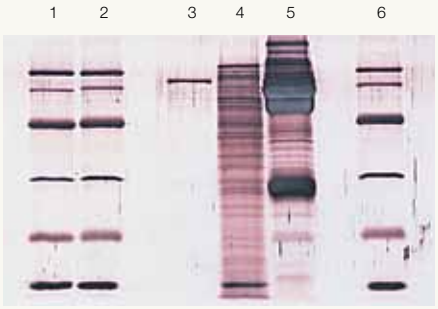


Fig. 4. Total protein staining of western blots. Colloidal gold staining of blot. Lane 1, low molecular weight standards; lanes 2 and 6, biotinylated standards; lane 3, human transferrin; lane 4, *E. coli* lysate; lane 5, total human serum.

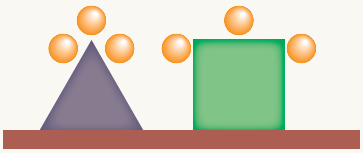


Fig. 5. Deposition-based total protein stains. All proteins on the blot bind dye or gold.

Stain	Detection Sensitivity	Assays Per Kit	Comments	Method	Results
Bio-Safe Coomassie	8–28 ng	50	• High background • Will not shrink membrane • Fast staining	• Compound binds to proteins to form colored bands	• Blue color on membrane
Colloidal gold	1 ng	100	• Rapid and very sensitive • Color does not fade • Will not shrink membrane • Optional enhancement increases sensitivity	• Compound binds to proteins to form colored bands	• Red color on membrane
SYPRO Ruby protein blot	2–8 ng	10–40	• Mass spectrometry compatible • UV fluorescent detection system required • Sensitive	• Compound binds to protein to give off fluorescent signal	• Fluorescent signal detected by epi-UV illumination

Ordering Information

Catalog #	Description
Total Protein Stains	
170-6527	**Colloidal Gold Total Protein Stain**, 500 ml
170-3127	**SYPRO Ruby Protein Blot Stain**, 200 ml
161-0786	**Bio-Safe Coomassie Stain**, 1 L

Colorimetric Detection

A Full Spectrum of Choices

Several substrates can be converted to a colored precipitate by enzymes such as HRP or AP. As the precipitate accumulates on the blot, a visible band develops (Figure 6). The enzyme reaction can be monitored and stopped when the desired signal over background is observed. Colorimetric detection is easier to perform than film-based detection methods, which must be developed by trial and error and use costly X-ray film and darkroom chemicals.

Colorimetric detection is typically considered a medium-sensitivity method compared with radioactive or chemiluminescent detection. However, Bio-Rad has amplified colorimetric systems that offer very high sensitivity comparable to that of chemiluminescent detection (Figure 7).

Immun-Blot HRP and AP Kits

The Immun-Blot assay kits provide the essential reagents to perform colorimetric detection on western blots with the added convenience of premixed buffers and enzyme substrates. Select your preferred combination of binding conjugates and color detection reagents. Available conjugates include AP- or HRP-conjugated secondary antibodies and HRP-conjugated protein A or protein G. Detection reagents include 4-chloro-1-naphthol (4CN) for HRP detection and 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium (BCIP/NBT) for AP detection. All kit components are individually quality-control tested in blotting applications. Included in each kit is an instruction manual with a thoroughly tested protocol and a troubleshooting guide, which simplify immunological detection and ensure excellent results.

Opti-4CN Substrate and Detection Kits

Opti-4CN is a formulation of 4CN that provides the same low-background results as standard 4CN but with much greater sensitivity and no more steps or reagents. Opti-4CN is available as a premixed substrate kit or combined with an HRP-conjugated antibody in a detection kit.

Amplified Opti-4CN Substrate and Detection Kits

The amplified Opti-4CN detection kits add further sensitivity to colorimetric blotting. Based on novel HRP-activated amplification reagents from Bio-Rad, colorimetric assays can now reach or surpass sensitivity levels previously available only with radioactivity or chemiluminescence, without the cost or time involved in darkroom development of blots (Figure 7).

Detection Method	Substrate	Detection Sensitivity	Signal Color	Product Options			Advantages
				Immun-Blot Assay Kits	Detection and Substrate Kits	Dry Powder	
Colorimetric HRP	4CN	500 pg	Purple	X	X	X	Fast color development, low cost, low background enzyme activity
Colorimetric HRP	DAB	500 pg	Brown			X	Insoluble product, readily chelated with osmium tetroxide. Sensitivity can be enhanced further by addition of metals
Colorimetric HRP	Opti-4CN	100 pg	Purple	X			High sensitivity, nonfading color, low background
Colorimetric HRP	Amplified Opti-4CN	5 pg	Purple	X			Best sensitivity available — equal to chemilumine-scence; kit provides all needed components
Colorimetric AP	BCIP/NBT	100 pg	Purple	X	X	X	High sensitivity

Premixed Liquid Substrates and Powdered Reagents

Premixed enzyme substrate kits provide the same low background and specific, optimal results obtained with powdered substrates — without the extra steps of weighing the chromogen and mixing it into solution. In addition to offering convenience and reliability, these kits reduce exposure to the hazards of the powdered reagents used in color development of western blots. Available substrates include 4CN, BCIP, NBT, and diaminobenzidine (DAB).

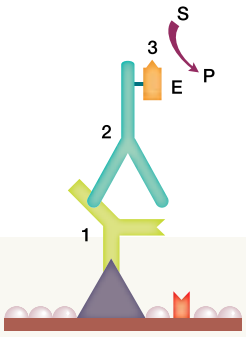


Fig. 6. General color detection system.
1. Antigen-specific primary antibody binds to protein of interest.
2. Enzyme-conjugated secondary antibody or binding protein binds to primary antibody.
3. Enzyme (E) converts substrate (S) to colored precipitate (P).

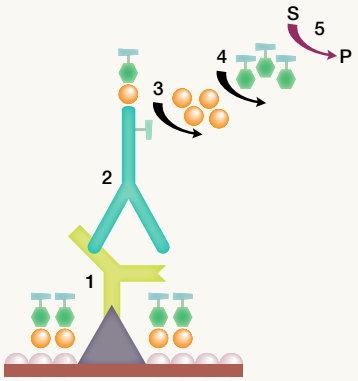


Fig. 7. Amplified Opti-4CN kit.
1. Antigen-specific primary antibody binds to the protein of interest.
2. HRP-conjugated secondary antibody binds to the primary antibody.
3. Amplification reagent reacts with HRP to incorporate biotin at the protein site.
4. Streptavidin-HRP binds to the incorporated biotin.
5. Enzyme converts substrate (S) to colored precipitate (P).

Ordering Information

Complete Blotting Kits **Immun-Blot Assay Kits** Each kit contains 10x TBS, Tween 20, gelatin blocker, and a secondary conjugate and substrate set.	Catalog # 170-6463 170-6464 170-6465 170-6460 170-6461 170-6462	Secondary Conjugate **Goat anti-rabbit-HRP** **Goat anti-mouse-HRP** **Goat anti-human-HRP** **Goat anti-rabbit-AP** **Goat anti-mouse-AP** **Goat anti-human-AP**	Substrate **4CN, HRP** **4CN, HRP** **4CN, HRP** **BCIP/NBT** **BCIP/NBT** **BCIP/NBT**
Opti-4CN Detection Kits Each kit contains a secondary conjugate and substrate set.	170-8237	**Goat anti-mouse-HRP**	**Opti-4CN**
Amplified Opti-4CN Detection Kits* Each kit contains 10x TBS, blocker, amplification reagent set, and a secondary conjugate and streptavidin-HRP substrate set.	170-8239 170-8240	**Goat anti-rabbit-HRP** **Goat anti-mouse-HRP**	**Opti-4CN** **Opti-4CN**

Colorimetric Substrates **Premixed Liquid Substrate Reagents** These ready-to-use colorimetric substrate solutions include buffer for convenient and fast blot detection.	Catalog # 170-8235 170-8238 170-6432 170-6431	Substrate **Opti-4CN substrate** **Amplified Opti-4CN substrate** **BCIP/NBT substrate** **4CN substrate**
Powdered Substrates The colorimetric substrates are supplied individually as dry powders for maximum shelf life.	170-6534 170-6539 170-6532 170-6535	**4CN**, 5 g **BCIP**, 300 mg **NBT**, 600 mg **DAB**, 5 g

Blotting Conjugates **Individual Blotting Grade Conjugates** These secondary antibodies and binding proteins are conjugated to an enzyme. The antibodies are isolated by affinity chromatography and then cross-adsorbed to eliminate nonspecific immunoglobulins.	Catalog # 170-6518 170-6520 170-6521 170-6515 170-6516 172-1050 170-6533 170-6528 170-3554 170-6522 170-6425	Conjugate **Goat anti-rabbit-AP**, 1 ml **Goat anti-mouse-AP**, 1 ml **Goat anti-human-AP**, 1 ml **Goat anti-rabbit-HRP**, 2 ml **Goat anti-mouse-HRP**, 2 ml **Goat anti-human-HRP**, 2 ml **Avidin-AP**, 1 ml **Avidin-HRP**, 2 ml **Streptavidin-AP**, 0.5 ml **Protein A-HRP**, 1 ml **Protein G-HRP**, 1 ml

* The secondary conjugate and substrate set are not premixed.

Protein Standards for Western Blotting

Ensure Reliable Blotting Results

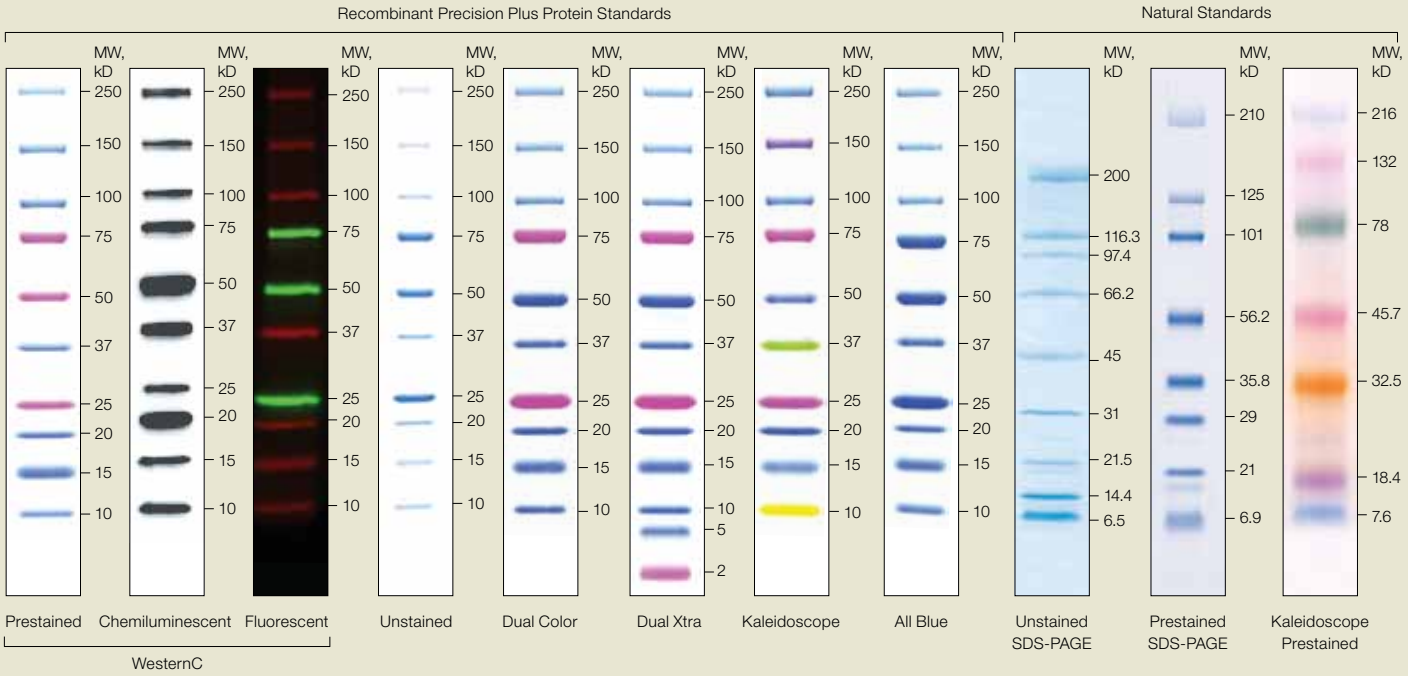
Bio-Rad offers a variety of protein standards for blotting applications. Precision Plus Protein™ WesternC™ standards have ten prestained bands engineered to contain a *Strep*-tag that enables chemiluminescent detection when probed with StrepTactin conjugates, so the protein standard appears on the gel, on the blot, and on the film or CCD image. The Precision Plus Protein Unstained standards also contain a *Strep*-tag for on-blot detection (for more information, request bulletins 2847 and 5576). Precision Plus Protein prestained standards can be used for molecular weight estimation and to assess transfer efficiency.

For more information on protein standards, see the current Life Science Research product catalog, or visit us on the Web at **discover.bio-rad.com/pppstandards**.

Blotting Standard Selection Guide

Product Name	Features	Applications
Precision Plus Protein WesternC standards	• Prestained multicolored fluorescent bands • Integrated *Strep*-tag for chemiluminescent visualization	• Monitoring electrophoresis • Monitoring blot transfer • Chemiluminescent detection • Multiplex fluorescent blot detection • Molecular weight determination
Precision Plus Protein Unstained standards	• Integrated *Strep*-tag for chemiluminescent visualization	• Chemiluminescent detection
Precision Plus Protein Dual Color standards	• Prestained multicolored fluorescent bands • 2-color band pattern	• Monitoring electrophoresis • Monitoring blot transfer • Multiplex fluorescent blot detection
Precision Plus Protein Dual Xtra standards	• Prestained multicolored fluorescent bands • 2-color band pattern • Extended MW range	• Monitoring electrophoresis • Monitoring blot transfer • Multiplex fluorescent blot detection
Precision Plus Protein™ Kaleidoscope™ standards	• Prestained multicolored fluorescent bands • 5-color band pattern	• Monitoring electrophoresis • Monitoring blot transfer • Multiplex fluorescent blot detection
Precision Plus Protein All Blue standards	• Prestained fluorescent bands	• Monitoring electrophoresis • Monitoring blot transfer • Fluorescent blot detection
Prestained SDS-PAGE standards (natural)	• Prestained bands (with fluorescence properties)	• Monitoring electrophoresis • Monitoring blot transfer • Fluorescent blot detection
Precision Plus Protein Kaleidoscope prestained standards (natural)	• Prestained multicolored fluorescent bands • Multicolor band pattern	• Monitoring electrophoresis • Monitoring blot transfer

Bio-Rad Offers a Variety of Standards for Western Blotting Applications



Ordering Information

Catalog #	Description	MW Range, kD
Unstained and Prestained Standards		
161-0376	**Precision Plus Protein WesternC Standards**, 250 µl, 50 applications	10–250
161-0399	**Precision Plus Protein WesternC Standards Value Pack**, 1.25 ml, 250 applications	10–250
161-0385	**Precision Plus Protein WesternC Pack**, 50 applications	10–250
161-0398	**Precision Plus Protein WesternC Pack Value Pack**, 250 applications	10–250
161-0363	**Precision Plus Protein Unstained Standards**, 1 ml, 100 applications	10–250
161-0396	**Precision Plus Protein Unstained Standards Value Pack**, 5 ml, 500 applications	10–250
161-0374	**Precision Plus Protein Dual Color Standards**, 500 µl, 50 applications	10–250
161-0394	**Precision Plus Protein Dual Color Standards Value Pack**, 2.5 ml, 250 applications	10–250
161-0377	**Precision Plus Protein Dual Xtra Standards**, 500 µl, 50 applications	2–250
161-0397	**Precision Plus Protein Dual Xtra Standards Value Pack**, 250 applications	2–250
161-0375	**Precision Plus Protein Kaleidoscope Standards**, 50 applications	10–250
161-0395	**Precision Plus Protein Kaleidoscope Standards Value Pack**, 2.5 ml, 250 applications	10–250
161-0373	**Precision Plus Protein All Blue Standards**, 500 µl, 50 applications	10–250
161-0393	**Precision Plus Protein All Blue Standards Value Pack**, 2.5 ml, 250 applications	10–250
161-0305	**SDS-PAGE Prestained Standards**, low range, 500 µl	20–103
161-0309	**SDS-PAGE Prestained Standards**, high range, 500 µl	48–204
161-0318	**SDS-PAGE Prestained Standards**, broad range, 500 µl	7.1–209
161-0324	**Kaleidoscope Prestained Standards**, 500 µl	7–216
Accessory Reagents		
170-6528	**Avidin-HRP**, 2 ml	
170-6533	**Avidin-AP**, 1 ml	
StrepTactin Conjugates		
161-0380	**Precision Protein™ StrepTactin-HRP Conjugate**, 300 µl, 150 applications	
161-0382	**Precision Protein StrepTactin-AP Conjugate**, 300 µl, 150 applications	

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Canada *905 364 3435* ***China*** *86 21 6169 8500* ***Czech Republic*** *420 241 430 532* ***Denmark*** *44 52 10 00* ***Finland*** *09 804 22 00*
France *01 47 95 69 65* ***Germany*** *089 31 884 0* ***Greece*** *30 210 777 4396* ***Hong Kong*** *852 2789 3300* ***Hungary*** *36 1 459 6100* ***India*** *91 124 4029300*
Israel *03 963 6050* ***Italy*** *39 02 216091* ***Japan*** *03 6361 7000* ***Korea*** *82 2 3473 4460* ***Malaysia*** *60 3 2117 5260* ***Mexico*** *52 555 488 7670*
The Netherlands *0318 540666* ***New Zealand*** *64 9 415 2280* ***Norway*** *23 38 41 30* ***Poland*** *48 22 331 99 99* ***Portugal*** *351 21 472 7700*
Russia *7 495 721 14 04* ***Singapore*** *65 6415 3170* ***South Africa*** *27 861 246 723* ***Spain*** *34 91 590 5200* ***Sweden*** *08 555 12700*
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