

# Western Blotting Detection Reagents

Maximize Western Blot Detection



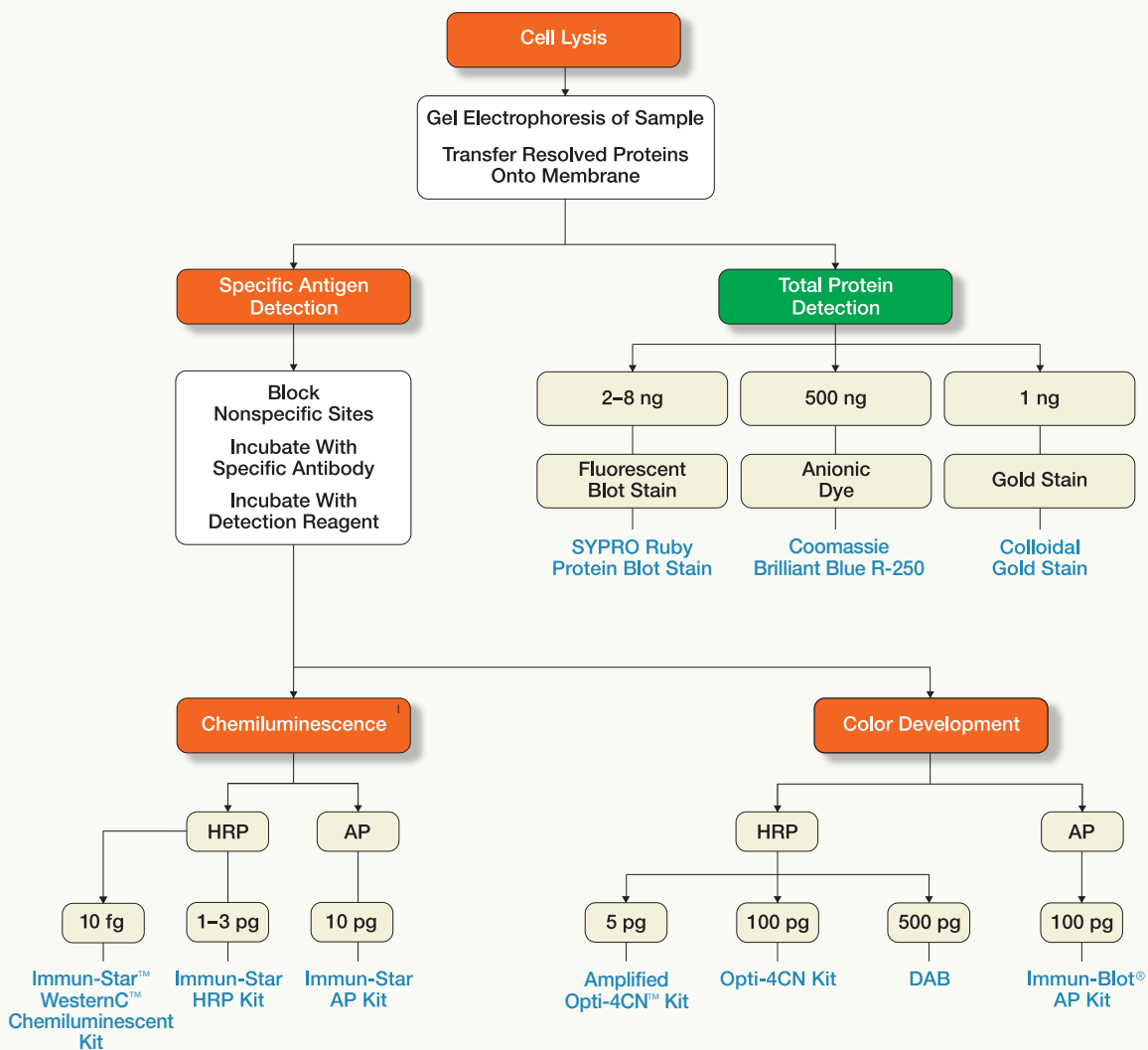
# Solutions for Any Blotting Application

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 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 horseradish peroxidase (HRP) or alkaline phosphatase (AP) secondary antibody conjugates, which can be used to generate a visible signal.



Blot detection reagent selection guide.



# Chemiluminescent Western Blot Detection

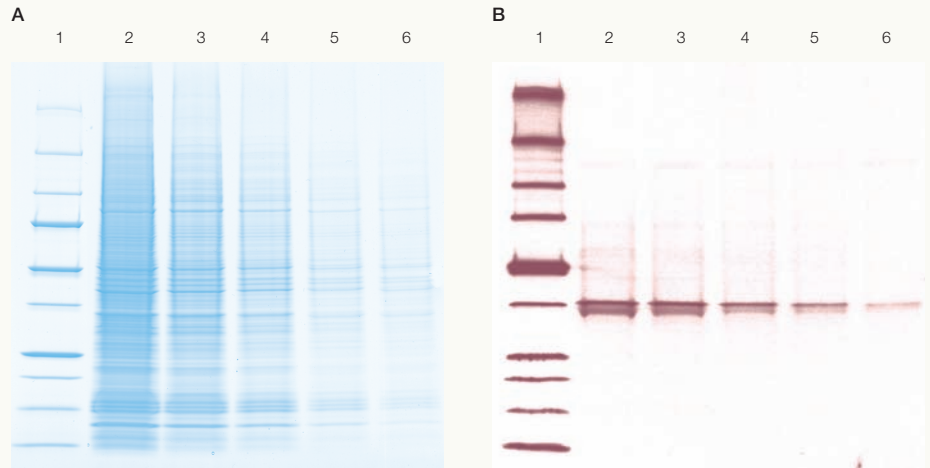
Superior Sensitivity

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 result (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 chemiluminescent 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	<ul style="list-style-type: none"> <li>• Conjugates</li> <li>• HRP substrate</li> <li>• Immun-Blot kits</li> </ul>	<ul style="list-style-type: none"> <li>• Short (30 sec) exposure</li> <li>• Signal duration 6–8 hr</li> <li>• Compatible with PVDF and nitrocellulose</li> <li>• Working solution stable for 24 hr at room temperature</li> </ul>	<ul style="list-style-type: none"> <li>• Azide inhibits enzyme activity</li> </ul>
Chemiluminescent AP	CDP- <i>Star</i>	10 pg	<ul style="list-style-type: none"> <li>• Conjugates</li> <li>• AP substrate</li> <li>• Immun-Blot kits</li> </ul>	<ul style="list-style-type: none"> <li>• 30 sec to 5 min exposure</li> <li>• Signal continues for 24 hr after activation</li> <li>• Blot can be reactivated</li> </ul>	<ul style="list-style-type: none"> <li>• Detects endogenous phosphatase activity, which may lead to false positives</li> </ul>

## Immun-Star WesternC Chemiluminescent 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 chemiluminescent 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 chemiluminescent kit for CCD imaging can expect improved quantitative results within each experiment compared with what can be obtained by film-based systems.



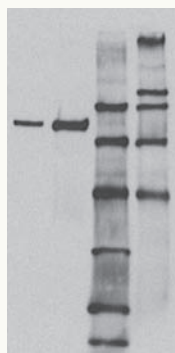
### Comparison of Immun-Star Chemiluminescent Kits

	Immun-Star HRP Kits	Immun-Star AP Kits	Immun-Star WesternC Kit
Sensitivity	1–3 pg	10 pg	5 fg
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

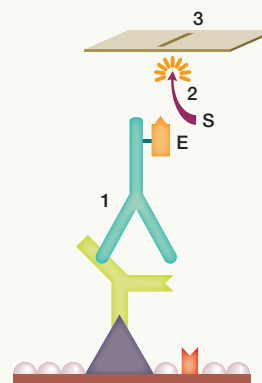
\* 1 mg/ml starting concentration.

### 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 chemiluminescent substrate CDP-Star produces the light signal (Figures 2 and 3). Blots can be reactivated, even weeks later, with addition of fresh substrate.



**Fig. 2. Detection of transferrin using the Immun-Star AP chemiluminescent 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
<b>Immun-Star HRP Kits and Components</b>							
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	•	•	–	–	–	–
170-5042	Goat Anti-Rabbit (GAR)-HRP Detection Reagents	•	•	–	–	–	–
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	–	•	–	–	–	–
<b>Immun-Star AP Kits and Components*</b>							
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	•	–	–	–	–	–
<b>Immun-Star WesternC Chemiluminescence Kit</b>							
170-5070	<b>Immuno-Star WesternC Chemiluminescence Kit</b> , includes 50 ml of luminal/enhancer, 50 ml of stable peroxide buffer, enough for 1,000 cm <sup>2</sup> of membrane						

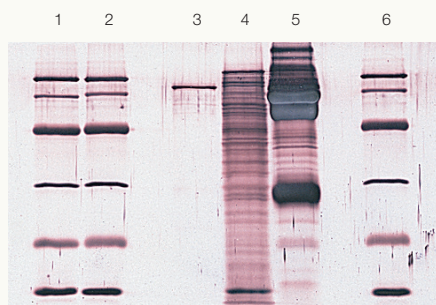
\* All items cover 2,500 cm<sup>2</sup> of membrane. Combine the blotting reagents pack with a detection kit to form a complete blotting system. Enhancer is used on nitrocellulose blots, but in most cases not on PVDF blots.

# Total Protein Stains

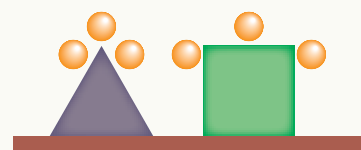
Discover the Pattern

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	<ul style="list-style-type: none"> <li>• High background</li> <li>• Will not shrink membrane</li> <li>• Fast staining</li> </ul>	<ul style="list-style-type: none"> <li>• Compound binds to proteins to form colored bands</li> </ul>	<ul style="list-style-type: none"> <li>• Blue color on membrane</li> </ul>
Colloidal gold	1 ng	100	<ul style="list-style-type: none"> <li>• Rapid and very sensitive</li> <li>• Color does not fade</li> <li>• Will not shrink membrane</li> <li>• Optional enhancement increases sensitivity</li> </ul>	<ul style="list-style-type: none"> <li>• Compound binds to proteins to form colored bands</li> </ul>	<ul style="list-style-type: none"> <li>• Red color on membrane</li> </ul>
SYPRO Ruby protein blot	2–8 ng	10–40	<ul style="list-style-type: none"> <li>• Mass spectrometry compatible</li> <li>• UV fluorescent detection system required</li> <li>• Sensitive</li> </ul>	<ul style="list-style-type: none"> <li>• Compound binds to protein to give off fluorescent signal</li> </ul>	<ul style="list-style-type: none"> <li>• Fluorescent signal detected by epi-UV illumination</li> </ul>

## 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

Enzymes such as HRP or AP convert several substrates to a colored precipitate. 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 to radioactive or chemiluminescent detection. However, Bio-Rad has amplified colorimetric systems that offer very high sensitivity comparable to 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).

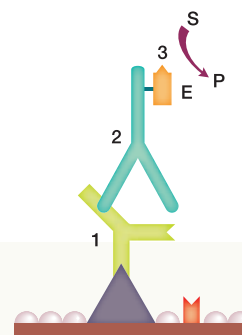
Detection Method	Substrate	Detection Sensitivity	Signal Color	Product Options	Advantages	Disadvantages
Colorimetric HRP	4CN	500 pg	Purple	<ul style="list-style-type: none"> <li>Dry powder, liquid substrate, Immun-Blot kits</li> </ul>	<ul style="list-style-type: none"> <li>Fast color development, low cost, low background enzyme activity</li> <li>Fast color development, precautions than for other substrates</li> <li>Azide inhibits enzyme activity</li> <li>High sensitivity, nonfading color, low background</li> <li>Best sensitivity available; unamplified protocol</li> </ul>	<ul style="list-style-type: none"> <li>Results fade over time; azide inhibits</li> <li>More safety</li> <li>More expensive than 4CN</li> <li>More steps than</li> </ul>
	DAB	500 pg	Brown	<ul style="list-style-type: none"> <li>Dry powder low background</li> </ul>		
	Opti-4CN	100 pg	Purple	<ul style="list-style-type: none"> <li>Liquid substrate, Opti-4CN kit</li> </ul>		
	Amplified Opti-4CN	5 pg	Purple	<ul style="list-style-type: none"> <li>Amplified Opti-4CN kit no extra materials (such as X-ray film) needed</li> </ul>		
Colorimetric AP	BCIP/NBT	100 pg	Purple	<ul style="list-style-type: none"> <li>Dry powder, liquid substrate, Immun-Blot kits</li> </ul>	<ul style="list-style-type: none"> <li>Sensitivity, stable storage of data</li> </ul>	<ul style="list-style-type: none"> <li>Detects endogenous phosphatase activity</li> </ul>



## Premixed Liquid Substrates and Powdered Reagents

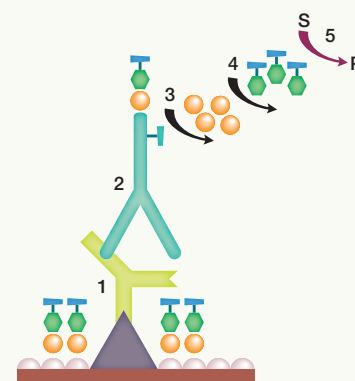
Premixed enzyme substrate kits provide the same low background and specific, optimal results provided by powdered substrates — but without the extra steps of weighing the chromogen and mixing

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).



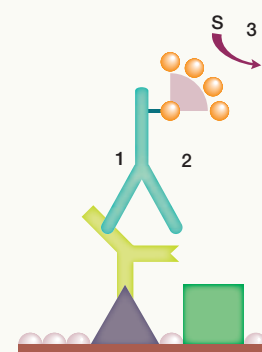
**Fig. 6. General color detection system.**

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).



**Fig. 8. Immun-Blot amplified AP kit.**

1. Biotinylated secondary antibody binds to primary antibody.
2. Complex of streptavidin and biotinylated-AP binds to biotin of secondary antibody.
3. Multiple APs are available to convert 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.

### Opti-4CN Detection Kits

Each kit contains a secondary conjugate and substrate set.

### Amplified Opti-4CN Detection Kits

Each kit contains 10x TBS, blocker, amplification reagent set, streptavidin-HRP, and a secondary conjugate and substrate set.

### Colorimetric Substrates

#### Premixed Liquid Substrate Reagents

These ready-to-use colorimetric substrate solutions include buffer for convenient and fast blot detection.

#### Powdered Substrates

The colorimetric substrates are supplied individually as dry powders for maximum shelf life.

### 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 #	Secondary Conjugate	Substrate
170-6463	Goat anti-rabbit-HRP	4CN, HRP
170-6464	Goat anti-mouse-HRP	4CN, HRP
170-6465	Goat anti-human-HRP	4CN, HRP
170-6460	Goat anti-rabbit-AP	BCIP/NBT
170-6461	Goat anti-mouse-AP	BCIP/NBT
170-6462	Goat anti-human-AP	BCIP/NBT
170-8237	Goat anti-mouse-HRP	Opti-4CN
170-8239	Goat anti-rabbit-HRP	Opti-4CN
170-8240	Goat anti-mouse-HRP	Opti-4CN

Catalog #	Substrate
170-8235	Opti-4CN substrate
170-8238	Amplified Opti-4CN substrate
170-6432	BCIP/NBT substrate
170-6431	4CN substrate
170-6534	4CN, 5 g
170-6539	BCIP, 300 mg
170-6532	NBT, 600 mg
170-6535	DAB, 5 g

Catalog #	Conjugate
170-6518	Goat anti-rabbit-AP, 1 ml
170-6520	Goat anti-mouse-AP, 1 ml
170-6521	Goat anti-human-AP, 1 ml
170-6515	Goat anti-rabbit-HRP, 2 ml
170-6516	Goat anti-mouse-HRP, 2 ml
172-1050	Goat anti-human-HRP, 2 ml
170-6533	Avidin-AP, 1 ml
170-6528	Avidin-HRP, 2 ml
170-3554	Streptavidin-AP, 0.5 ml
170-6522	Protein A-HRP, 1 ml
170-6425	Protein G-HRP, 1 ml

# 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 chemiluminescence 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](http://discover.bio-rad.com/pppstandards).

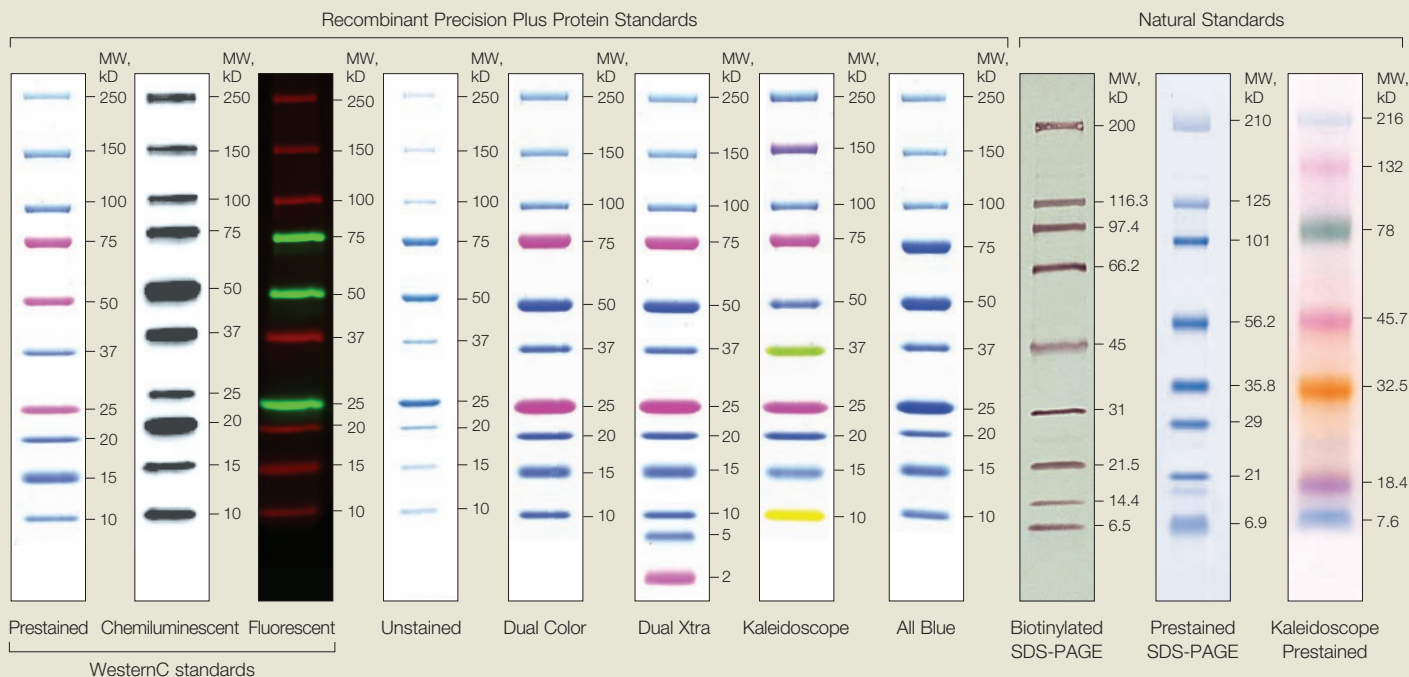
## Blotting Standard Selection Guide

Product Name	Features	Applications
Precision Plus Protein WesternC standards	<ul style="list-style-type: none"><li>• Prestained multicolored fluorescent bands</li><li>• Integrated <i>Strep</i>-tag for chemiluminescent visualization</li></ul>	<ul style="list-style-type: none"><li>• Monitoring electrophoresis</li><li>• Monitoring blot transfer</li><li>• Chemiluminescent detection</li><li>• Multiplex fluorescent detection</li></ul>
Precision Plus Protein unstained standards	<ul style="list-style-type: none"><li>• Integrated <i>Strep</i>-tag for chemiluminescent visualization</li></ul>	<ul style="list-style-type: none"><li>• Chemiluminescent detection unstained standards: 161-0363</li><li>• Precision Plus Protein unstained standard plugs: 161-0378</li></ul>
Precision Plus Protein Dual Color standards	<ul style="list-style-type: none"><li>• Prestained multicolored fluorescent bands</li><li>• 2 color band pattern</li></ul>	<ul style="list-style-type: none"><li>• Monitoring electrophoresis</li><li>• Monitoring blot transfer</li><li>• Multiplex fluorescent detection</li></ul>
Precision Plus Protein Dual Xtra standards	<ul style="list-style-type: none"><li>• Prestained multicolored fluorescent bands</li><li>• 2 color band pattern</li><li>• Extended MW range</li></ul>	<ul style="list-style-type: none"><li>• Monitoring electrophoresis</li><li>• Monitoring blot transfer</li><li>• Multiplex fluorescent detection</li></ul>
Precision Plus Protein™ Kaleidoscope™ standards	<ul style="list-style-type: none"><li>• Prestained multicolored fluorescent bands</li><li>• 5 color band pattern</li></ul>	<ul style="list-style-type: none"><li>• Monitoring electrophoresis</li><li>• Monitoring blot transfer</li><li>• Multiplex fluorescent detection</li></ul>
Precision Plus Protein All Blue standards	<ul style="list-style-type: none"><li>• Prestained fluorescent bands</li></ul>	<ul style="list-style-type: none"><li>• Monitoring electrophoresis</li><li>• Monitoring blot transfer</li></ul>
Biotinylated standards	<ul style="list-style-type: none"><li>• Biotinylated for avidin HRP or AP binding</li></ul>	<ul style="list-style-type: none"><li>• Detection on blot</li></ul>
Prestained SDS-PAGE standards	<ul style="list-style-type: none"><li>• Prestained bands</li></ul>	<ul style="list-style-type: none"><li>• Monitoring electrophoresis</li><li>• Monitoring blot transfer</li></ul>





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



### Ordering Information

Catalog #	Description	MW Range, kD
<b>Unstained and Prestained Standards</b>		
161-0376	Precision Plus Protein WesternC Standards, 50 applications	10–250
161-0385	Precision Plus Protein WesternC Pack, includes 50 applications of WesternC standards and 50 applications of Streptactin-HRP	10–250
161-0363	Precision Plus Protein Unstained Standards, 1 ml, 100 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
161-0375	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
161-0325	Kaleidoscope Polypeptide Standards, broad range, 500 µl	3.8–36.4

### Biotinylated SDS-PAGE Standards

161-0311	Biotinylated SDS-PAGE Standards, high range, 250 µl	45–200
161-0306	Biotinylated SDS-PAGE Standards, low range, 250 µl	14.4–97.4
161-0319	Biotinylated SDS-PAGE Standards, broad range, 250 µl	6.5–200
161-0312	Biotinylated SDS-PAGE Standards Kit, high range, avidin-HRP	45–200
161-0313	Biotinylated SDS-PAGE Standards Kit, high range, avidin-AP	45–200
161-0307	Biotinylated SDS-PAGE Standards Kit, low range, avidin-HRP	14.4–97.4
161-0308	Biotinylated SDS-PAGE Standards Kit, low range, avidin-AP	14.4–97.4
161-0321	Biotinylated SDS-PAGE Standards Kit, broad range, avidin-HRP	6.5–200
161-0322	Biotinylated SDS-PAGE Standards Kit, broad range, avidin-AP	6.5–200

### Accessory Reagents

170-6528	Avidin-HRP, 2 ml
170-6533	Avidin-AP, 1 ml

### StrepTactin Conjugates

161-0380	Precision Protein StrepTactin-HRP Conjugate, 0.3 ml, 150 applications
161-0382	Precision Protein StrepTactin-AP Conjugate, 0.3 ml, 150 applications

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