Strep-tag Technology for Molecular Weight (MW) Determinations on Blots Using Precision Plus Protein™ Standards

Introduction

Bio-Rad has consistently provided innovative standards for electrophoresis and blotting. In 2002, Precision Plus Protein* standards were designed to produce the sharpest and most dependable unstained and prestained standards for gel electrophoresis and western blotting. These protein standards are a blend of ten recombinant proteins that have precise molecular weights ranging from 10 to 250 kD. They are available in three versions: unstained, all blue, and dual color.

Precision Plus Protein standards offer several advantages over traditional protein electrophoresis standards. First, they contain protein bands that migrate at even, consistent MW increments (10, 15, 20, 25, 37, 50, 75, 100, 150, and 250 kD).

A second important feature is that all three versions — unstained, all blue, and dual color — comigrate and show crisp, tight bands when electrophoresed (Figure 1). Comigration and alignment of the standards allow more accurate MW determinations with prestained standards.

All three versions of the Precision Plus Protein standards have three reference bands, at 25, 50, and 75 kD. These proteins are present at higher concentrations than the other bands to provide instant gel orientation (Figure 1).

Finally, each unstained protein band has been engineered to contain a unique affinity *Strep*-tag peptide, which allows detection and MW determination on western blots.

Immunoblotting and MW Determination

Electrophoresis of proteins on polyacrylamide gels is often followed by transfer of proteins to a membrane and immunological detection of the proteins using standard western blotting techniques. The presence of the *Strep*-tag affinity peptide on each protein in the unstained Precision Plus Protein standards allows for simple, convenient, and precise MW determinations directly on blotted membranes (Schmidt and Skerra 1993). The *Strep*-tag affinity tag sequence is a short stretch of amino acids located either internally or on the C-terminus of each recombinant protein (Schmidt et al. 1996). The *Strep*-tag peptide has intrinsic binding affinity towards both native streptavidin and StrepTactin, a genetically modified form of streptavidin. The high-affinity binding of StrepTactin to the *Strep*-tag peptide allows convenient detection of proteins on western blots.

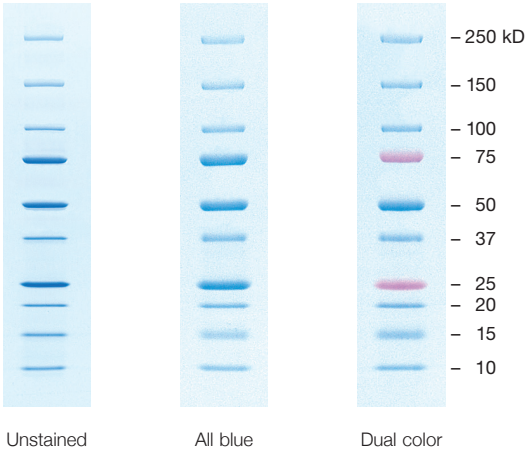

Fig. 1. Comigration of prestained and unstained Precision Plus Protein standards. All standards were run on a 4–20% Tris-HCl Criterion™ precast gel, and the unstained standards were stained with Coomassie Blue.

Overview of the StrepTactin Detection System

The detection of unstained Precision Plus Protein standards, followed by accurate MW determination directly on western blots, is a straightforward procedure that utilizes conventional blotting methods and reagents (Figure 2). The StrepTactin-AP or -HRP conjugate can be incorporated into any blotting protocol without adding any additional steps or unusual reagents. After electrophoresis, the standards with their *Strep*-tag sequence are transferred to a membrane, which is then blocked with the desired blocking agent. The blot is probed with the primary antibody of interest, washed, and then probed simultaneously with the secondary antibody and StrepTactin, both of which contain the same enzyme conjugate. The standards, along with the antigen of interest, are visualized by the addition of the appropriate colorimetric or chemiluminescent AP or HRP substrate (Figure 3). Once the proteins are visualized, the distance migrated by each protein can be used to construct a standard curve. The MW of an unknown protein can then be obtained from the standard curve (Voss and Skerra 1997).

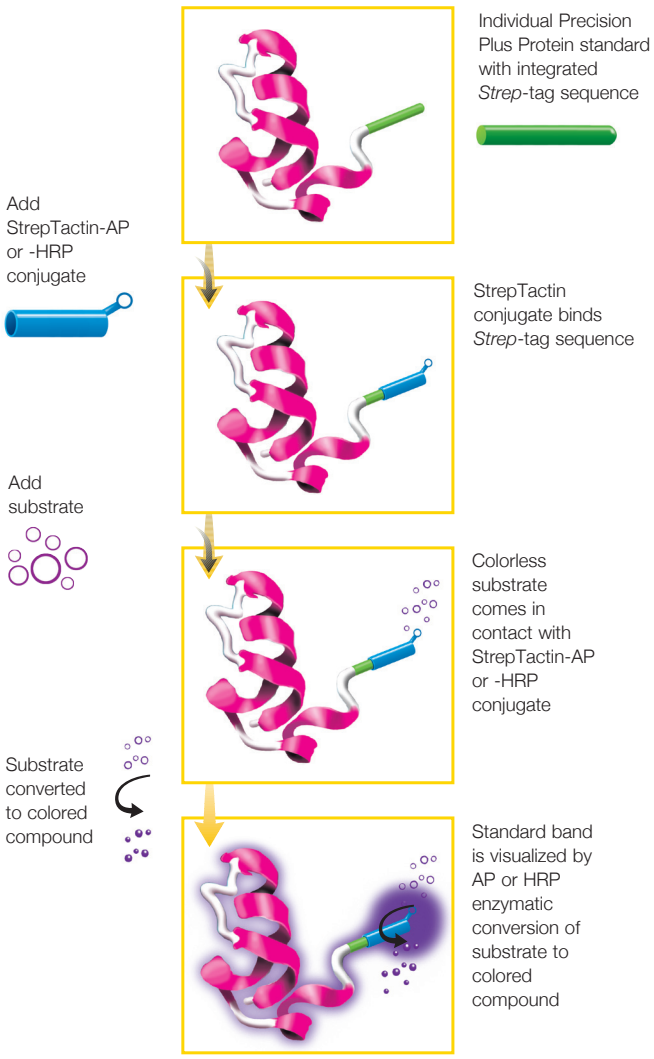

Fig. 2. Overview of the StrepTactin detection system.

StrepTactin, a Modified Form of Streptavidin

StrepTactin is an engineered form of streptavidin that displays optimized binding to the *Strep*-tag affinity tag (Voss and Skerra 1997). Compared to streptavidin, the engineered StrepTactin is a superior tool for detection of proteins on western blots. StrepTactin, as opposed to streptavidin, can be used in conjunction with a wide range of blocking and dilution buffers, including BSA, gelatin, casein, and nonfat dry milk. Because the use of BSA and gelatin results in higher sensitivity in band detection, they are the recommended blocking agents. If casein or nonfat dry milk is used in blocking and dilution buffers, the final development times need to be lengthened slightly to increase the signal. StrepTactin binds to all ten proteins of the unstained Precision Plus Protein standards with equal affinity. Conversely, streptavidin detects higher-MW proteins more strongly than lower-MW proteins, making development of bands on the blot more problematic. Finally, when probing complex lysates, nonspecific detection of endogenous biotin carrier proteins can also be problematic (Schmidt and Skerra 1993). However, StrepTactin conjugates are less cross-reactive with the 22 kD *E. coli* biotin carrier protein compared to streptavidin conjugates. Thus, preblocking the blot with avidin is not always necessary with StrepTactin conjugates, as it is with streptavidin. For these reasons, StrepTactin conjugates should always be used to achieve optimum detection of unstained Precision Plus Protein standards and for optimum performance in western blots.

Advantages of the Precision Plus Protein Standards/ StrepTactin Detection System

MW determination on western blots using the Precision Plus Protein standards/*Strep*-tag/StrepTactin detection system is far superior to traditional methods. Commonly used approaches include the transfer of prestained standards, or the transfer of unstained standards followed by Ponceau S staining of the blot. The downsides to these methods include the inaccuracy in migration of traditional prestained standards and the reversibility of the Ponceau S stain. Another approach involves the transfer of biotinylated standards and detection with streptavidin-AP or -HRP conjugates. The biotinylated standards provide accurate MW information on blots, but require an additional dilution step compared to the *Strep*-tag/StrepTactin detection system.

The use of unstained Precision Plus Protein standards for MW determination on western blots overcomes all of the disadvantages of traditional systems. Unstained standards are detected as crisp, evenly spaced, accurate-MW bands directly on western blots. These versatile standards, with their integrated *Strep*-tag sequence, can be used for either colorimetric or chemiluminescent detection systems. Finally, unstained Precision Plus Protein standards come prediluted and ready to load for both AP- and HRP-based detection. Thus, a single tube of standards can be used for Coomassie Blue-stained gels, silver-stained gels, and colorimetric or chemiluminescent detection on blots.

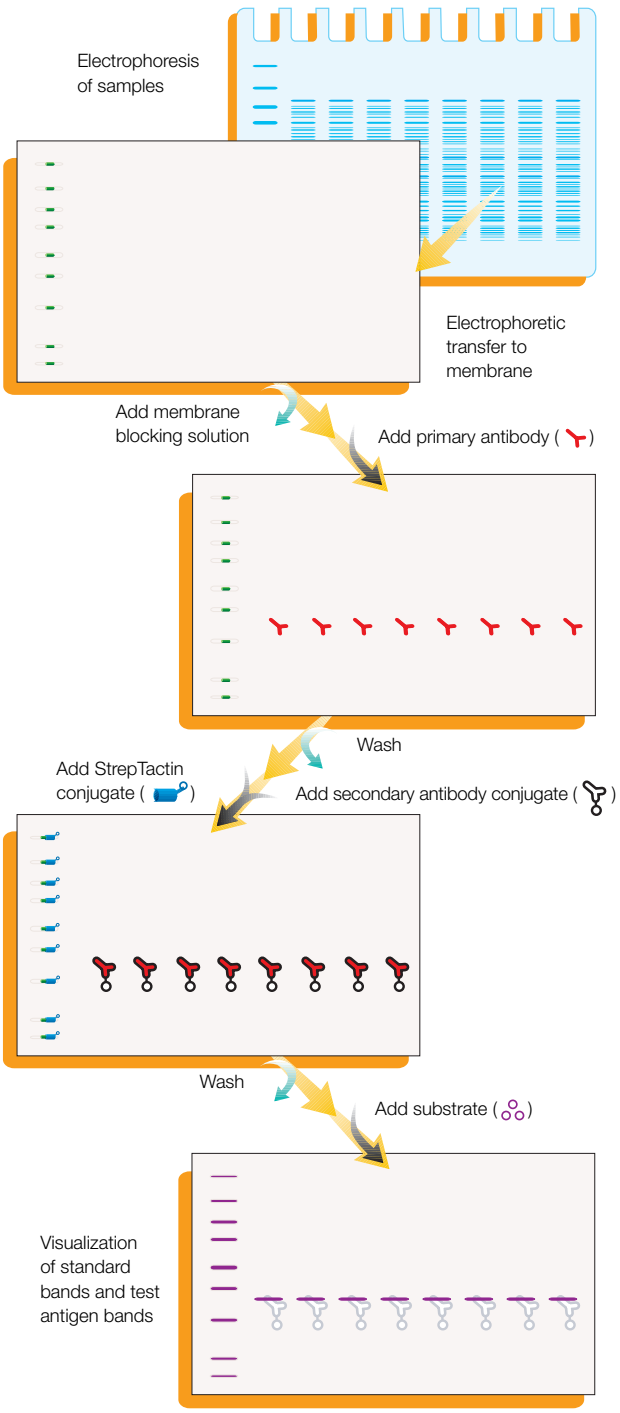

Fig. 3. Summary of Precision Plus Protein standard band detection on an intact blot.

Standard Detection When Probing for an Overexpressed Protein

To prevent overexposure of unstained Precision Plus Protein standard bands on blots, the level of the antigen of interest that will be detected with the primary antibody must be considered. If an overexpressed or highly purified protein is meant to be detected, then the standards lane can be left attached to the blot of interest. The standards can be used with any conventional blotting protocol, requiring no additional time-consuming steps. The StrepTactin conjugate is conveniently added along with the secondary conjugated antibody, and development of the standard bands can parallel detection of the other proteins of interest (Figure 3). An example of this method of detection is shown in Figure 4, where an overexpressed GFP fusion protein is detected in an *E. coli* lysate. Detection was performed with the Opti-4CN™ detection kit (catalog #170-8237); a more detailed description of the protocol can be found in the Precision Plus Protein manual. Note the specific detection of the GFP fusion protein (49 kD), which illustrates the specificity of the StrepTactin conjugates for the standard bands. Although the membrane was not blocked with avidin, nonspecific detection of the biotin carrier protein was not observed. Protein load (Precision Plus Protein standards and cell lysate), detection system, and development time can all be customized to optimally detect the standards and the target antigens of interest.

Recommendations for Opti-4CN Detection

Loading volume, Precision Plus Protein unstained standards	5 µl, 1:10 dilution
StrepTactin-HRP conjugate	1:5,000 dilution
Blot development time	15 min

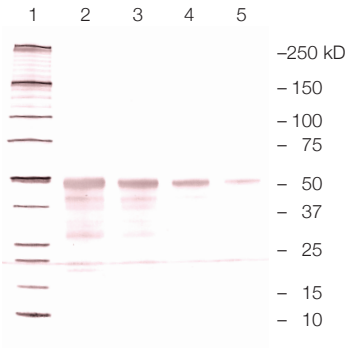

Fig. 4. Opti-4CN detection of Precision Plus Protein unstained standard bands and a GFP fusion protein. Unstained standards (lane 1) and a dilution series of *E. coli* lysate (lanes 2–5) containing high levels of GFP fusion protein were electrophoresed on a 4–20% Criterion gel and transferred to a nitrocellulose membrane. The intact gel was probed with an antibody to GFP, and then incubated with StrepTactin-HRP and goat anti-mouse-HRP. The blot was developed using the Opti-4CN kit.

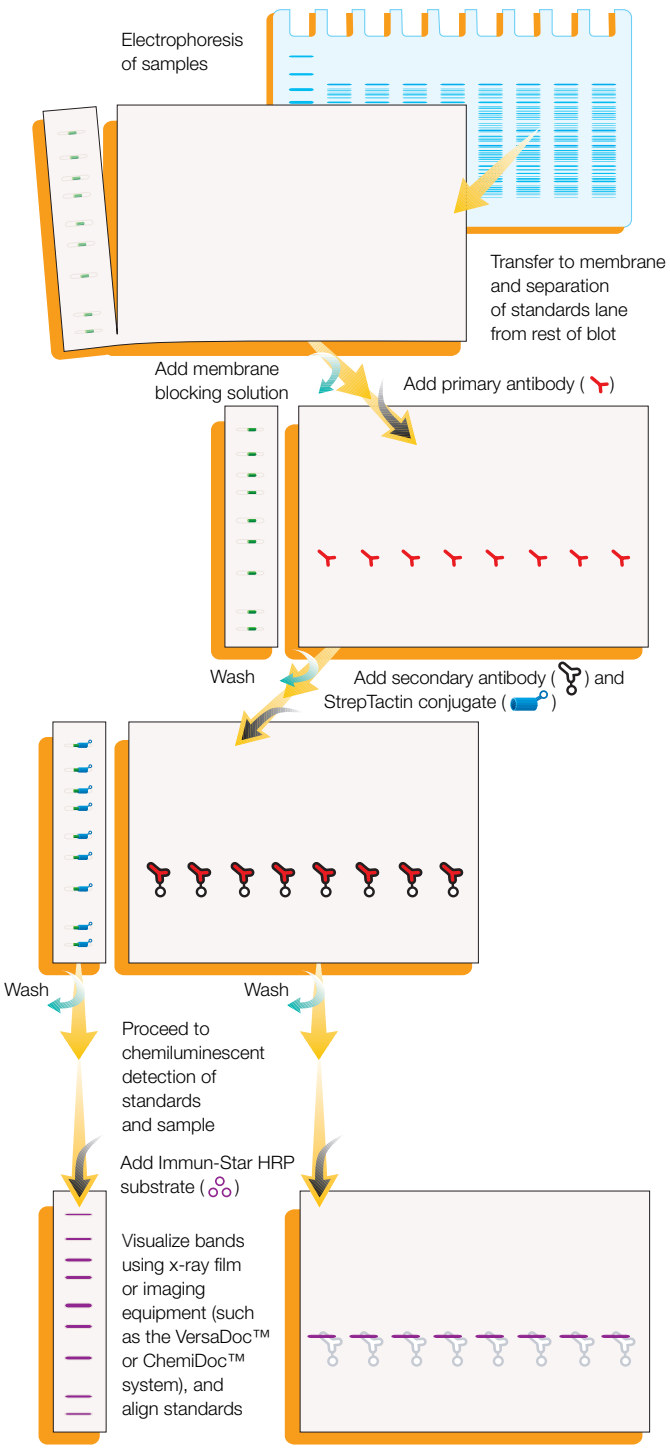

Fig. 5. Summary of Precision Plus Protein standard band detection in conjunction with low-level antigen detection.

Standard Detection When Probing for a Low-Level Protein

When the antigen of interest is expressed at a very low level relative to other proteins in a complex lysate, Precision Plus Protein standards can be used for MW determination by either of two methods. The lane containing the standards can be separated from the main blot prior to development, as shown in Figure 5. This method will allow independent optimal development of both the standards and the antigen of interest from a single blotting experiment. Alternatively, the appropriate amount of the standards to load to ensure comparable signal intensities from the standards and the antigen of interest can be determined in advance using a dilution series. This method allows direct analysis of protein mobilities without the need to separate the blot into two portions that must later be realigned. Either of these methods permits accurate MW analysis of proteins expressed even at very low levels.

The gel and blot in Figure 6 show an experiment that required maximum detection sensitivity to visualize low levels of a human cyclin-dependent kinase (CDK7) protein from a HeLa cell lysate dilution series. After incubation with a primary antibody specific for CDK7, the blot was probed with an HRP-conjugated secondary antibody as well as with the StrepTactin-HRP conjugate (catalog #161-0380). The Immun-Star™ HRP chemiluminescent kit (catalog #170-5040) was used to detect both CDK7 and the Precision Plus Protein unstained standards. A detailed description of the one-step protocol can be found in the manual that accompanies the Immun-Star kit.

Recommendations for Immun-Star HRP Chemiluminescent Detection

Loading volume, Precision Plus Protein unstained standards	0.2–5 µl
StrepTactin-HRP conjugate	1:5,000 dilution
Development time for standards (0.5 µl)	5–30 sec

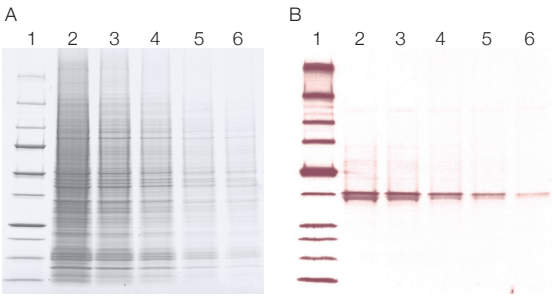

Fig. 6. Detection of CDK7 protein and Precision Plus Protein unstained standards using the Immun-Star HRP chemiluminescent detection kit. A, 10 µl of standards (lane 1) and a dilution series of a HeLa cell lysate (lanes 2–6) were electrophoresed on a 4–20% Criterion gel. The gel was stained with Bio-Safe™ Coomassie stain (catalog #161-0786) 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 the StrepTactin-HRP conjugate. After a 2 min incubation in the Immun-Star HRP detection solution, the blot was exposed to film for 5 sec.

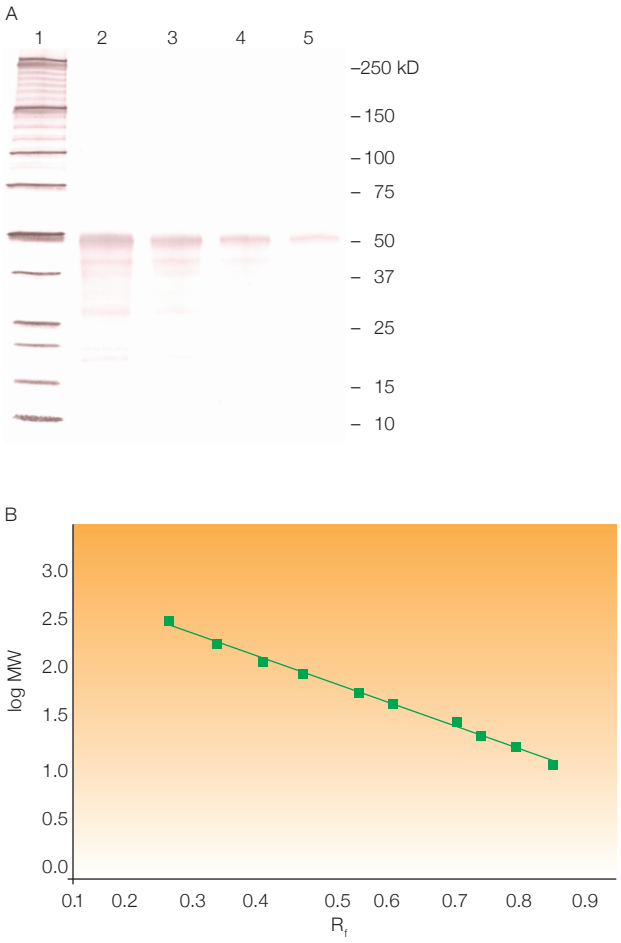

Fig. 7. Alkaline phosphatase-based detection of Precision Plus Protein standard bands and MW determination. A, Precision Plus Protein unstained standards (lane 1) and a dilution series of an *E. coli* lysate (lanes 2–5) containing low levels of a GFP fusion protein were electrophoresed on a 4–20% Criterion gel and transferred to a membrane. The blot was probed with antibodies and detection conjugates and colorimetrically developed with the Immun-Blot AP conjugate substrate kit. B, the R_f of each standard band was plotted against its log MW to construct a standard curve.

Standard Detection When Intermediate Sensitivity Is Required

For experiments that require intermediate levels of sensitivity, StrepTactin-AP conjugates can be used with Immun-Blot® colorimetric detection kits (catalog #170-6460, 170-6461, and 170-6462). Figure 7A illustrates the detection of low levels of a GFP fusion protein in an *E. coli* lysate. After colorimetric detection of the standard bands and unknown GFP fusion proteins, the relative mobility of each standard band was determined. The data generated were used to construct a standard curve (Figure 7B), and the MW of the GFP fusion protein (~49 kD) was calculated based upon its migration distance and the data from the standard curve (Voss and Skerra 1997). The experimental protocol was identical to that of the schematic in Figure 3.

Recommendations for Immun-Blot AP Detection

Loading volume, Precision Plus Protein standards	5 µl, 1:10 dilution
StrepTactin-HRP conjugate	1:5,000 dilution
Blot development time	5 min

Conclusions

Bio-Rad's new Precision Plus Protein standards with their integrated *Strep*-tag peptide allow accurate and simple MW determinations of protein antigens directly on western blots, using either colorimetric or chemiluminescent detection methods. The unstained Precision Plus Proteins standards can be used with any standard blotting protocol, and the StrepTactin detection system requires no additional or unusual steps.

References

Schmidt TGM and Skerra A, The random peptide library-assisted engineering of a C-terminal affinity peptide, useful for the detection and purification of a functional Ig Fv fragment, Protein Eng 6, 109–122 (1993)

Schmidt TGM et al., Molecular interaction between the *Strep*-tag affinity peptide and its cognate target streptavidin, J Mol Biol 255, 753–766 (1996)

Voss S and Skerra A, Mutagenesis of a flexible loop in streptavidin leads to higher affinity for the *Strep*-tag II peptide and improved performance in recombinant protein purification, Protein Eng 10, 975–982 (1997)

Ordering Information

Criterion Tris-HCl Gels

(All gels have a 4% stacking gel except 4–15% and 4–20%)	12+2 Comb 45 µl Samples	18-Well Comb 30 µl Samples	26-Well Comb 15 µl Samples
5% Gel	345-0001	345-0002	345-0003
7.5% Gel	345-0005	345-0006	345-0007
10% Gel	345-0009	345-0010	345-0011
12.5% Gel	345-0014	345-0015	345-0016
15% Gel	345-0019	345-0020	345-0021
18% Gel	345-0023	345-0024	345-0025
4–15% Gradient Gel	345-0027	345-0028	345-0029
4–20% Gradient Gel	345-0032	345-0033	345-0034
8–16% Gradient Gel	345-0037	345-0038	345-0039
10–20% Gradient Gel	345-0042	345-0043	345-0044

Catalog #	Description

Precision Plus Protein Standards and StrepTactin Conjugates

161-0373	Precision Plus Protein All Blue Standards
161-0374	Precision Plus Protein Dual Color Standards
161-0363	Precision Plus Protein Unstained Standards
161-0380	Precision Protein™ StrepTactin-HRP Conjugate, 0.3 ml
161-0382	Precision Protein StrepTactin-AP Conjugate, 0.3 ml

Electrophoresis Reagents

161-0737	Laemmli Sample Buffer, 30 ml
161-0772	10x Tris/Glycine/SDS Electrophoresis Buffer, 5 L cube
161-0787	Bio-Safe Coomassie Stain, 5 L cube

Blotting Reagents

170-8236	Opti-4CN Detection Kit, goat anti-rabbit (GAR)-HRP
170-8237	Opti-4CN Detection Kit, goat anti-mouse (GAM)-HRP
170-8239	Amplified Opti-4CN Detection Kit, GAR-HRP
170-8240	Amplified Opti-4CN Detection Kit, GAM-HRP
170-6460	Immun-Blot Kit, GAR-AP
170-6461	Immun-Blot Kit, GAM-AP
170-6463	Immun-Blot Kit, GAR-HRP
170-6464	Immun-Blot Kit, GAM-HRP
170-6432	AP Conjugate Substrate Kit
170-6431	HRP Conjugate Substrate Kit
161-0734	10x Tris/Glycine Transfer Buffer, 5 L cube
170-6435	10x Tris-Buffered Saline (TBS), 1 L
161-0781	10% Tween 20, 1 L
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
170-5045	Goat Anti-Rabbit (GAR)-HRP Detection Kit
170-5047	Goat Anti-Mouse (GAM)-HRP Conjugate
170-5046	Goat Anti-Rabbit (GAR)-HRP Conjugate

Catalog #	Description

Blotting Membranes and Filter Paper

162-0112	Nitrocellulose Membrane (0.45 µm), 33 cm x 3 m roll
162-0115	Nitrocellulose Membrane (0.2 µm), 33 cm x 3 m roll
162-0167	Nitrocellulose Membrane (0.45 µm), 8.5 x 13.5 cm, 10
162-0168	Nitrocellulose Membrane (0.2 µm), 8.5 x 13.5 cm, 10
162-0070	Supported Nitrocellulose Membrane (0.45 µm), 8.5 x 13.5 cm, 10
162-0071	Supported Nitrocellulose Membrane (0.2 µm), 8.5 x 13.5 cm, 10
170-3956	Thick Blot Absorbent Filter Paper, 15 x 20 cm, 25 sheets

Blotting Membrane Filter Paper Sandwich (7 x 8.5 cm)*

162-0212	0.2 µm Nitrocellulose Filter Paper Sandwich, 20 pack
162-0213	0.2 µm Nitrocellulose Filter Paper Sandwich, 50 pack
162-0214	0.45 µm Nitrocellulose Filter Paper Sandwich, 20 pack
162-0215	0.45 µm Nitrocellulose Filter Paper Sandwich, 50 pack
162-0216	0.2 µm PVDF Filter Paper Sandwich, 20 pack
162-0217	0.2 µm PVDF Filter Paper Sandwich, 50 pack
162-0218	Immun-Blot PVD/Filter Paper Sandwich, 7 x 8.5 cm, 20 pack
162-0219	Immun-Blot PVD/Filter Paper Sandwich, 7 x 8.5 cm, 50 pack

Blotting Membrane Filter Paper Sandwich (8.5 x 13.5 cm)*

162-0232	0.2 µm Nitrocellulose Filter Paper Sandwich, 20 pack
162-0233	0.2 µm Nitrocellulose Filter Paper Sandwich, 50 pack
162-0234	0.45 µm Nitrocellulose Filter Paper Sandwich, 20 pack
162-0235	0.45 µm Nitrocellulose Filter Paper Sandwich, 50 pack
162-0236	0.2 µm PVDF Filter Paper Sandwich, 20 pack
162-0237	0.2 µm PVDF Filter Paper Sandwich, 50 pack
162-0238	Immun-Blot PVD/Filter Paper Sandwich, 8.5 x 13.5 cm, 20 pack
162-0239	Immun-Blot PVD/Filter Paper Sandwich, 8.5 x 13.5 cm, 50 pack

* Each sandwich consists of one membrane (nitrocellulose or PVDF) and 2 sheets of thick filter paper cut to fit Criterion precast gels (8.5 x 13.5 cm) or Ready Gel® precast gels (7 x 8.5 cm).

Equipment

165-6001	Criterion Cell, includes tank, lid with cables, 3 sample loading guides to match 12+2 well, 18-well, 26-well combs, instructions
170-4070	Criterion Blotter With Plate Electrodes, includes tank, lid with cables, 2 gel holder cassettes, filter paper (50 sheets), 4 fiber pads, gel blot assembly tray and roller, sealed ice cooling unit, instructions
170-4071	Criterion Blotter With Wire Electrodes, includes tank, lid with cables, 2 gel holder cassettes, filter paper (50 sheets), 4 fiber pads, gel blot assembly tray and roller, sealed ice cooling unit, instructions

BIO-RAD

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *www.bio-rad.com* ***USA*** *800 424 6723* ***Australia*** *61 2 9914 2800* ***Austria*** *01 877 89 01* ***Belgium*** *09 385 55 11* ***Brazil*** *55 31 3689 6600* ***Canada*** *905 364 3435* ***China*** *86 20 8732 2339* ***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* ***Mexico*** *52 555 488 7670* ***The Netherlands*** *0318 540666* ***New Zealand*** *0508 805 500* ***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 3188* ***South Africa*** *27 861 246 723* ***Spain*** *34 91 590 5200* ***Sweden*** *08 555 12700* ***Switzerland*** *061 717 95 55* ***Taiwan*** *886 2 2578 7189* ***United Kingdom*** *020 8328 2000*