

## Related Products for Blot Detection

Catalog #	Description
161-0380	<b>Precision Protein StrepTactin-HRP Conjugate</b> , 300 $\mu$ l
161-0381	<b>Precision Protein StrepTactin-HRP Conjugate</b> , 125 $\mu$ l
161-0382	<b>Precision Protein StrepTactin-AP Conjugate</b> , 300 $\mu$ l
170-5060	<b>Clarity Western ECL Substrate</b> , 200 ml
170-5061	<b>Clarity Western ECL Substrate</b> , 500 ml
170-5046	<b>Immun-Star GAR-HRP Conjugate</b> , 2 ml
170-5047	<b>Immun-Star GAM-HRP Conjugate</b> , 2 ml
170-5010	<b>Immun-Star GAM-AP Detection Kit</b>
170-5011	<b>Immun-Star GAR-AP Detection Kit</b>

*Strep*-tag and StrepTactin are trademarks of Institut für Bioanalytik GmbH. *Strep*-tag technology for western blot detection is covered by US patent 5,506,121 and by UK patent 2,272,698. StrepTactin is covered by German patent application P 19641876.3. Bio-Rad Laboratories, Inc. is licensed by Institut für Bioanalytik GmbH to sell these products for research use only.

Precision Plus Protein standards are sold under license from Life Technologies Corporation, Carlsbad, CA, for use only by the buyer of the product. The buyer is not authorized to sell or resell this product or its components.

For more information, go to [bio-rad.com](http://bio-rad.com).

## Precision Plus Protein™ WesternC™ Standards Instruction Manual

### Precision Plus Protein WesternC Standards

Single unit, catalog #161-0376

5-pack value pack, catalog #161-0399

### Precision Plus Protein WesternC Pack

Single unit, catalog #161-0385

5-pack value pack, catalog #161-0398

Product shipped at room temperature.

Store at  $-20^{\circ}\text{C}$  for 12 months.



## Product Description

Precision Plus Protein WesternC Standards offer 10 distinct, prestained protein bands with a molecular weight of 10, 15, 20, 25, 37, 50, 75, 100, 150, and 250 kD. The 25, 50, and 75 kD bands are stained pink for easy band referencing and blot orientation.

Precision Plus Protein WesternC Standards contain an integral *Strep*-tag sequence, enabling parallel detection on western blots using Precision Protein StrepTactin-HRP Conjugate (catalog #161-0380, 161-0381) or Precision Protein StrepTactin-AP Conjugate (catalog #161-0382) and HRP- or AP-conjugated secondary antibodies specific to the protein of interest.

The molecular weights of the Precision Plus Protein WesternC Standards are confirmed by migration in a Laemmli SDS-PAGE system. The sharp ladder bands provide an accurate estimation of the molecular weight of unknown proteins, with an  $r^2$  value >0.99 on a 4–20% Tris-HCl SDS-PAGE gel.\*

## Instructions for Use

Precision Plus Protein WesternC Standards are provided in loading buffer and require no dilution. Allow the tube to reach room temperature and thoroughly mix before use. This will ensure that any solids that may have precipitated have returned to solution. Do not heat the product above room temperature.

For chemiluminescent detection of the Precision Plus Protein WesternC Standards

1. Load 5  $\mu$ l of the Precision Plus Protein WesternC Standard onto the gel next to protein of interest.
2. Run the SDS-PAGE gel at the appropriate voltage, current, and time.
3. Transfer proteins to a membrane at the appropriate voltage and current.
4. Block the membrane in blocking solution.
5. Probe with a primary antibody specific to the protein of interest.
6. Wash the blot.

\*Migration pattern of the 25, 50, and 75 kD bands may differ on neutral pH gels (Criterion XT Gels or similar).

7. Probe with a secondary antibody appropriate to the protein of interest, and include approximately 1  $\mu$ l of StrepTactin-HRP conjugate<sup>1</sup> or StrepTactin-AP conjugate per 10 ml of secondary incubation solution to detect the bands of the Precision Plus Protein WesternC Standards.
8. Wash the blot.
9. Develop with a suitable development reagent, such as Bio-Rad's Clarity™ Western ECL Substrate or Bio-Rad's Immun-Star™ AP Detection Kit.

Note: The recommended dilution of the StrepTactin-HRP and StrepTactin-AP conjugate is 1:10,000–1:50,000 for chemiluminescent detection<sup>2</sup> with the Clarity Substrate.

Note: As with every use of new antibodies or conjugates, it is important to test for cross reactivity of samples (i.e., a negative control where the StrepTactin-HRP or AP conjugate is added without the primary antibody to the target protein). It is known that StrepTactin-HRP and AP react with biotin carboxyl carrier protein (22.5 kD) in *E. coli* extracts. Generally, biotinylated proteins can be sufficiently masked by adding 50  $\mu$ g/ml avidin to the blocking solution.

## Recommended Loading Volumes

Load Volume <sup>3</sup>	Application
5 $\mu$ l	Chemiluminescent blot development

## Specifications

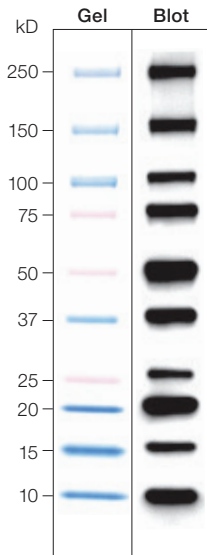
Catalog #	161-0376, single unit 161-0399, 5-pack value pack 161-0385, single unit 161-0398, 5-pack value pack
Volume	250 $\mu$ l
Number of applications	50, single unit 250, 5-pack value pack
Loading buffer	30% (w/v) glycerol, 2% SDS, 62.5 mM Tris, pH 6.8, 50 mM DTT, 5 mM EDTA, 0.02% Na <sub>2</sub> S <sub>2</sub> O <sub>8</sub> , 0.01% bromophenol blue
Shelf life	Store at –20°C for 12 months.

<sup>1</sup> StrepTactin-HRP conjugates and StrepTactin-AP conjugates are designed to not interfere with secondary antibody incubation.

<sup>2</sup> For longer exposure times, use of a greater dilution of StrepTactin-HRP (1:50,000–1:100,000) will prevent overexposure of the bands of the Precision Plus Protein WesternC Standards.

<sup>3</sup> For very short exposure times, or for enhanced band intensity on a gel or a blot, an optional load volume of 10  $\mu$ l may be used.

A representative lot of Precision Plus Protein WesternC Standards on a 4–20% Criterion™ Tris-HCl Gel and a western blot detected by chemiluminescence



**Precision Plus Protein Standards** are the latest generation of recombinant proteins designed to provide a ladder of convenient, consistent sizes. These standards continue the tradition of accuracy and ease of use begun by Precision Protein™ Standards, while also providing instant orientation, even greater accuracy, particularly in the 10–25 kD range, and two-color convenience.

**Precision Plus Protein WesternC Standards** are intended for use in a wide range of applications and allow easy visualization during electrophoresis, blot transfer, and chemiluminescent development of western blots on film or by CCD imagers.