Ordering Information

Precision Protein Conjugates and Standards

Catalog #	Description
161-0380	Precision Protein StrepTactin-HRP Conjugate, 0.3 ml
161-0382	Precision Protein StrepTactin-AP Conjugate, 0.3 ml
161-0363	Precision Plus Protein Unstained Standards, 1.0 ml
161-0381	Precision Protein StrepTactin-HRP Conjugate, 0.125 ml
161-0376	Precision Plus Protein WesternC Standards, 0.25 ml

Blotting Reagents

170-6435	Premixed Tris Buffered Saline, 10x, 1 L
161-0782	1x TBS/1% Casein, 1 L
170-6537	Gelatin, blotting grade, 200 ml
170-6531	Tween 20, blotting grade, 100 ml
162-0115	Nitrocellulose Membrane (0.45 µm), 33 cm x 3 m
162-0112	Nitrocellulose Membrane (0.2 µm), 33 cm x 3 m
170-5040	Immun-Star HRP Substrate, 500 ml
170-5041	Immun-Star HRP Substrate, 100 ml
170-5070	Immun-Star WesternC Kit, 100 ml

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Instruction Manual

Precision Protein™ StrepTactin-HRP Conjugate

Catalog #161-0380
Catalog #161-0381

Precision Protein™ StrepTactin-AP Conjugate

Catalog #161-0382

BIO-RAD

Introduction

Precision Protein StrepTactin conjugates are specifically intended for use with Precision Plus Protein™ unstained standards (catalog #161-0363) and Precision Plus Protein™ WesternC™ standards (catalog #161-0376). These standards contain an integral *Strep*-tag amino acid sequence that is recognized by StrepTactin conjugates. StrepTactin is a modified form of streptavidin that binds with greater affinity and specificity to the *Strep*-tag sequence.

Precision Protein StrepTactin conjugates are labeled with either horseradish peroxidase (HRP) (catalog #161-0380, 161-0381) or with alkaline phosphatase (AP) (catalog #161-0382). The conjugates are compatible with chemiluminescent detection methods (Bio-Rad's Immun-Star™ system) and with colorimetric detection systems (Bio-Rad's Immun-Blot® system).

Specifications

	HRP Conjugate (161-0380, 161-0381)	AP Conjugate (161-0382)
Contents	0.3 ml/0.125 ml	0.3 ml
Number of applications	150 blots/60 blots	150 blots
Storage	4ºC	4°C
Shelf life	1 year at 4ºC	1 year at 4°C
Load volume of Precision Plus Protein unstained standards	1–6 µl colorimetric; 5–10 µl chemiluminescent*	1–4 µl colorimetric; 5–10 µl chemiluminescent**
Load volume of Precision Plus Protein WesternC standards	5–10 µl chemiluminescent	5–10 µl chemiluminescent
Recommended working dilution	Colorimetric Kits: 1:5000 Immun-Star HRP Kit: 1:10,000–1:15,000 Immun-Star WesternC Kit: 1:10,000–1:50,000	Colorimetric Kits: 1:5000 Immun-Star AP Kit: 1:5,000–1:10,000
Buffers	1x PBS, 5 mg/ml BSA, 0.01%, thimerosal, 3.5 mg/ml, StrepTactin-HRP	50 mM Tris, 0.1 M NaCl, 1 mM $MgCl_2$, 0.1% NaN_3, 10 mg/ml BSA, 0.5 mg/ml StrepTactin-AP

* 1:30 – 1:60 dilution of standards
** 1:15 – 1:30 dilution of standards

Abbreviated Procedure

For complete instructions, order any of the Immun-Blot assay kits or the Immun-Star chemiluminescent detection kits. Reading the entire instruction manual is advised for optimal results and avoidance of common problems inherent to western blotting procedures.

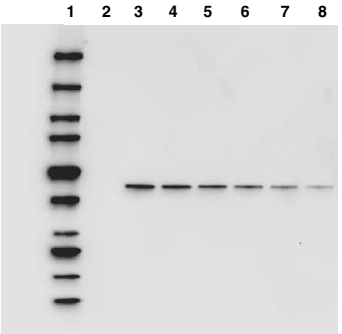
NOTE: All steps are to be done at room temperature. Wear gloves and protective clothing when preparing and working with the solutions in the assay.

1. Prepare the membrane blot, i.e., electro-phoretic blotting, passive blotting, or filter lifting. If the antigen of interest is present at very low levels, it is best to separate the Precision Plus Protein standards lane with the rest of the blot and perform the detection in parallel, or perform multiple exposures during chemiluminescent detection. This will avoid overdevelopment of the standards.
2. Block the membrane with blocking solutions of choice for 1 hr with gentle agitation (we recommend 3% BSA, 3% gelatin, or 1% casein; milk solutions have a high biotin content that tends to decrease the signal). To minimize detection of endogenous biotin carrier proteins, add 50 µg/ml avidin to the blocking solution.
3. Decant the blocking solution and wash the membrane with TTBS (0.05% Tween 20 in TBS) for 5 min with gentle agitation.
4. Decant the wash solution and incubate with primary antibody (diluted in TTBS) for 1 hr with gentle agitation.
5. Decant primary antibody solution and wash the membrane in TTBS for 5 min. Repeat twice for a total wash time of 15 min.
6. Decant the wash solution and add diluted StrepTactin conjugate (dilute according to the recommended working dilution for the kit you are using in TTBS) and the appropriate conjugated second-

ary antibody, diluted in the same buffer. Incubate for 1 hr with gentle agitation.

7. Decant the conjugate solution and wash in TTBS for 5 min. Repeat.
8. Perform a final 5 min wash in TBS to remove residual detergent.
9. Proceed to the development step (either the color development step if using Bio-Rad's Immun-Blot assay kits, or the blot development step if using Bio-Rad's Immun-Star kits.

   NOTE: When using chemiluminescent detection kits, exposure times may vary. To obtain optimal results, it may be necessary to separate the markers from the rest of the blot and expose them separately, orperform multiple exposures of the standards and unknown lanes.



Lane 1: Precision Plus Protein WesternC standards
Lane 2: Blank
Lanes 3–8: Actin, serial dilution, 200, 150, 100, 75, 50, 25 ng; detection used, Immun-Star WesternC chemiluminescent kit (catalog #170-5070)