

## Standards for **Electrophoresis and Blotting**

A Diverse Portfolio for All Applications





# A Diverse Portfolio of Standards

Bio-Rad's standards provide an excellent means of monitoring electrophoresis and blotting experiments. A variety of protein standards have been formulated for a large range of applications, including SDS-PAGE, western blotting, 2-D PAGE, and isoelectric focusing (IEF). These standards can be used for gel and blot orientation, to monitor transfer efficiency, and for molecular weight (MW) estimation and determination.

Standards can be categorized by application:

## SDS-PAGE and Western Blotting Standards

- Recombinant prestained protein standards
- Recombinant unstained protein standards
- Natural prestained SDS-PAGE protein standards
- Natural unstained SDS-PAGE protein standards

## Specialty Standards

- Protein standards for IEF
- Protein standards for 2-D SDS-PAGE

	Precision Plus Protein™ Standards						Prestained Natural Standards				Unstained Natural Standards				Specialty Standards		
	WesternC™	Kaleidoscope™	Dual Xtra	Dual Color	All Blue	Unstained	Broad Range	Low Range	High Range	Natural Kaleidoscope	Broad Range	Low Range	High Range	Polypeptide	IEF	2-D	Standard Plugs***
MW/pI range	10–250 kD	10–250 kD	2–250 kD	10–250 kD	10–250 kD	10–250 kD	6.9–210 kD	14–97 kD	45–200 kD	7.6–216 kD	6.5–200 kD	14–97 kD	45–200 kD	1.4–26.6 kD	4.45–9.6 pI	17.5–76 kD 4.5–8.5 pI	10–250 kD
Number of proteins	10	10	12	10	10	10	8	6	4	7	9	6	5	6	9	7	10
<b>Electrophoresis</b>																	
SDS-PAGE	•	•	•	•	•	•	•	•	•	•	•	•	•	•	-	-	•
Accurate MW estimation	•	•	•	•	•	•	-	-	-	-	•	•	•	•	-	-	•
Multicolored	•	•	•	•	-	-	-	-	-	•	-	-	-	-	-	-	-
Coomassie staining	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Fluorescent staining	-	-	-	-	-	•	-	-	-	-	•	•	•	•	-	•	•
2-D electrophoresis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	•	-
IEF	-	-	-	-	-	-	-	-	-	-	-	-	-	-	•	-	-
Plug format for use in gels with no reference well	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	•
<b>Blotting</b>																	
Monitoring transfer efficiency	•	•	•	•	•	-	•	•	•	•	-	-	-	-	-	-	-
Coomassie staining	•	•	•	•	•	•	•	•	•	•	•	•	•	•	-	•	•
Fluorescent staining	-	-	-	-	-	•	-	-	-	-	•	•	•	•	-	•	•
Fluorescent blotting*	•	•	•	•	•	-	-	-	-	-	-	-	-	-	-	-	-
Immunodetection**	•	-	-	-	-	•	-	-	-	-	-	-	-	-	-	-	•
<b>Catalog Numbers</b>																	
Single unit	161-0385	161-0375	161-0377	161-0374	161-0373	161-0363	161-0318	161-0305	161-0309	161-0324	161-0317	161-0304	161-0303	161-0326	161-0310	161-0320	161-0378
Value pack of 5 units	161-0398	161-0395	161-0397	161-0394	161-0393	161-0396	-	-	-	-	-	-	-	-	-	-	-

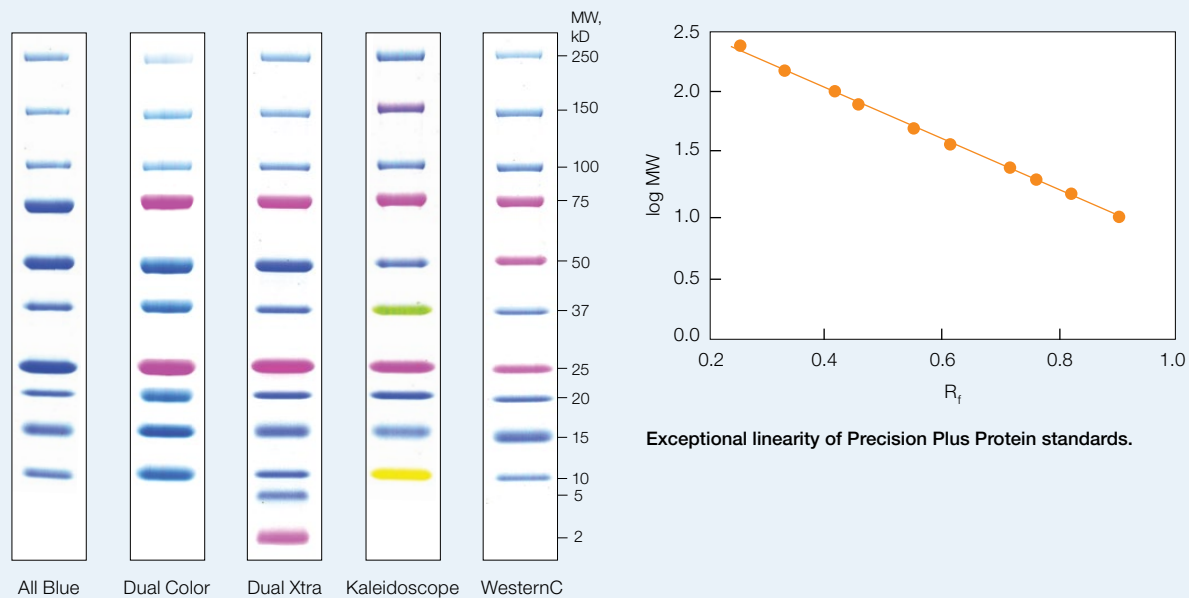
\* These standards have fluorescent properties and can be used for fluorescent blotting applications. See bulletin 5723 for details on using Precision Plus Protein WesternC standards for fluorescent multiplexing. Precision Plus Protein Dual Xtra standards (161-0377) are recommended for fluorescent blot analysis of proteins between 5 and 250 kD.  
 \*\* Immunodetection via addition of a Precision Protein™ StrepTactin and horseradish peroxidase (HRP) or StrepTactin and alkaline phosphatase (AP) conjugate, which will bind to the internal Strep-tags on the proteins.  
 \*\*\* 24 unstained plugs for 2-D gels.



## Recombinant Prestained Protein Standards

Bio-Rad offers a complete family of Precision Plus Protein prestained standards, including All Blue, Dual Color, Dual Xtra, Kaleidoscope, and WesternC options. All standards show the same pattern, with only minimal shift, and can be used for MW determination of unknown proteins in gels and on blots. Precision Plus Protein prestained standards offer:

- Exceptional linearity ( $R^2 > 0.99$ ) for determining MW
- 10 sharp, nonshifting bands (10–250 kD)
- Lot-to-lot consistency
- Matching migration patterns among the entire Precision Plus Protein standards family
- 3 high-intensity reference bands in the All Blue and Dual Color standards — at 25, 50, and 75 kD



Precision Plus Protein prestained standards family.

### New and Improved Precision Plus Protein Dual Color Standard

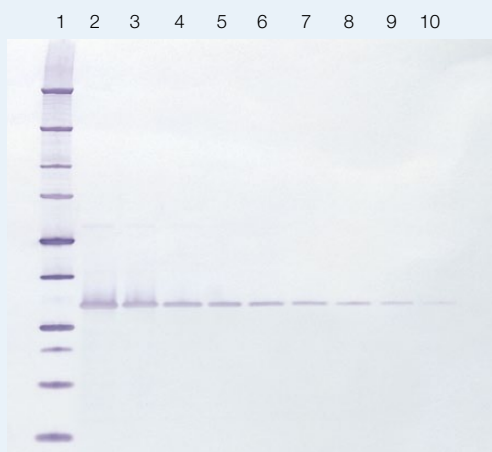
- Brighter for easy identification
- Sharper for accurate molecular weight estimation
- Stronger band intensity throughout blot development



## Recombinant Unstained Protein Standards

Recombinant unstained protein standards allow accurate MW determination on SDS-PAGE gels and western blots. Precision Plus Protein unstained standards have the following attributes:

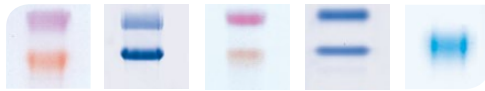
- Bands in every batch have the same MW, confirmed by mass spectrometry and migration in a Laemmli SDS-PAGE buffer system
- Unique *Strep*-tag affinity sequence allows detection and MW determination on western blots
- 10 sharp, nonshifting bands (10–250 kD)
- 3 high-intensity reference bands — at 25, 50, and 75 kD



**Western blot detection of green fluorescent protein (GFP) and Precision Plus Protein unstained standards using the Immun-Blot® AP colorimetric detection kit.** Maximum sensitivity achievable with Immun-Blot AP is 100 pg. A gel run with 4  $\mu$ l of standards (lane 1) and a dilution series of *E. coli* lysate containing overexpressed GFP (lanes 2–10) was transferred to a PVDF membrane. The blot was probed with a primary antibody specific for GFP, then incubated with StrepTactin-AP and a secondary antibody conjugated to AP. The blot was developed using the Immun-Blot AP kit.



**Western blot detection of GFP and Precision Plus Protein unstained standards using the Immun-Star™ HRP chemiluminescent detection kit.** Maximum sensitivity achievable with Immun-Star HRP is 1–3 pg. A gel run with 0.5  $\mu$ l of standards (lane 1) and a dilution series of *E. coli* lysate containing overexpressed GFP (lanes 2–8) was transferred to a PVDF membrane. The blot was probed with a primary antibody specific for GFP, then incubated with StrepTactin-HRP and a secondary antibody conjugated to HRP. The blot was developed using the Immun-Star HRP kit.



## Natural Prestained SDS-PAGE Protein Standards

Prestained standards are visualized before the gel is stained, making them ideal for monitoring protein migration during an electrophoretic run, for gel and blot orientation, and for assessing transfer efficiency.

### Prestained SDS-PAGE Standards

- Available in high, low, and broad ranges
- Blended proteins give uniform band intensities
- Covalently bound dye will not dissociate during normal staining or destaining

### Kaleidoscope Prestained Standards

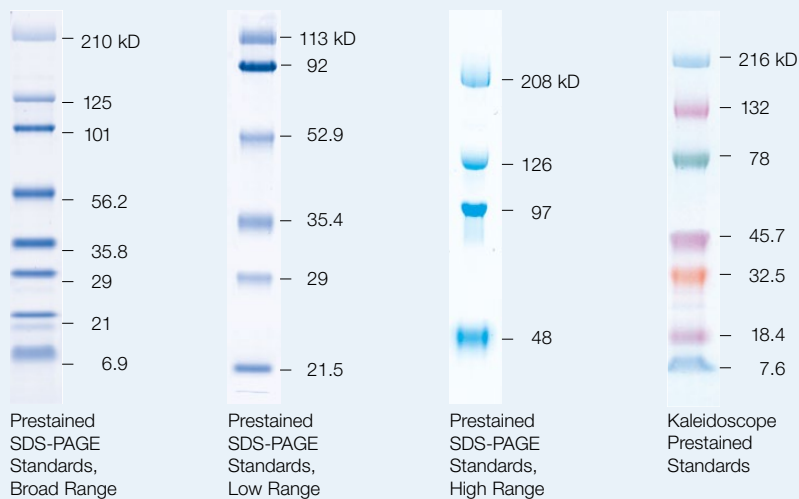
These standards include all attributes of the prestained SDS-PAGE standards, plus:

- Individually colored proteins for instant band recognition
- Good transfer efficiency for western blotting

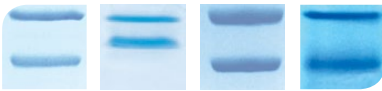
Constituent proteins of natural prestained SDS-PAGE standards.

Protein	Source	Approximate MW* (kD)	Prestained SDS-PAGE Standards			Kaleidoscope Standards
			Broad	Low	High	Prestained
Myosin	Rabbit skeletal muscle	200.0	•		•	•
β-Galactosidase	<i>E. coli</i>	116.3	•		•	•
Phosphorylase b	Rabbit muscle	97.4		•		
Serum albumin (BSA)	Bovine	66.2	•	•	•	•
Ovalbumin	Hen egg white	45.0	•	•	•	
Carbonic anhydrase	Bovine	31.0	•	•		•
Trypsin inhibitor	Soybean	21.5	•	•		•
Lysozyme	Hen egg white	14.4	•	•		•
Aprotinin	Bovine pancreas	6.5	•			•

\* MW will vary from lot to lot; see lot-specific calibration included with standards.



**Natural prestained standards.** Molecular weights shown are of representative lots. Actual weights may vary.



## Natural Unstained SDS-PAGE Protein Standards

Natural unstained protein standards allow accurate MW determination on SDS-PAGE gels. Every batch is tested for proper mobility, providing a reliable control for gel-to-gel variability.

### SDS-PAGE Standards

- Available in high, low, and broad MW ranges
- Blended to give uniform band intensities with Coomassie Blue R-250 stain

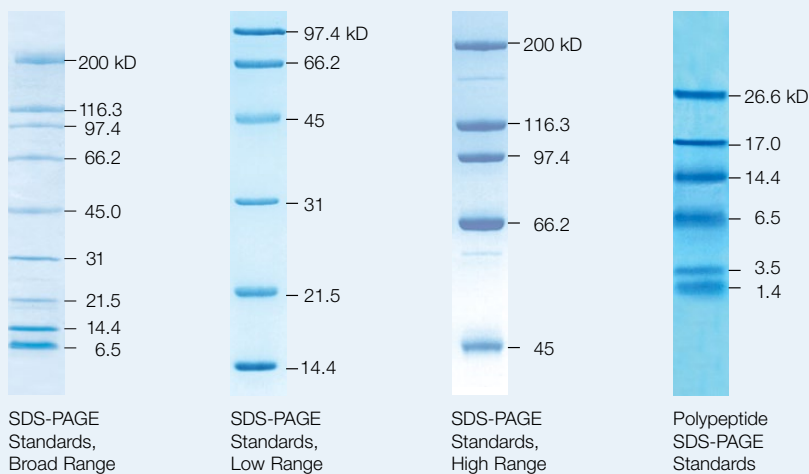
### Polypeptide SDS-PAGE Standards

- Formulated for MW determination of peptides and small proteins resolved on Tris-Tricine gels
- Contain 6 polypeptides with MW ranging from 1.4 to 26.6 kD
- Blended to stain uniformly with Coomassie G-250 stain

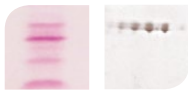
### Constituent proteins of natural unstained SDS-PAGE standards.

Protein	Source	MW (kD)	Ranges Available*			
			Broad	Low	High	Polypeptide
Myosin	Rabbit skeletal muscle	200.0	●		●	
β-Galactosidase	<i>E. coli</i>	116.3	●		●	
Phosphorylase b	Rabbit muscle	97.4	●	●	●	
Serum albumin	Bovine	66.2	●	●	●	
Ovalbumin	Hen egg white	45.0	●	●	●	
Carbonic anhydrase	Bovine	31.0	●	●		
Triosephosphate isomerase	Rabbit	26.6				●
Trypsin inhibitor	Soybean	21.5	●	●		
Myoglobin	Equine	17.0				●
α-Lactalbumin	Bovine	14.5				●
Lysozyme	Hen egg white	14.4	●	●		
Aprotinin	Bovine pancreas	6.5	●			●
Insulin B chain, oxidized	Bovine	3.5				●
Bacitracin	<i>Bacillus licheniformis</i>	1.4				●

\* SDS-PAGE — high, low, broad, and polypeptide.



Natural unstained standards.



## Specialty Protein Standards for IEF

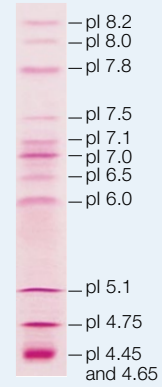
### IEF Standards

- Allow reproducible, dependable pI calibration in native polyacrylamide and agarose IEF gels
- Contain 9 native proteins with pI ranging from 4.45 to 9.6
- 5 of the 9 proteins are naturally colored to monitor focusing

### Constituent proteins of IEF standards.\*

Protein	Color	pI
Cytochrome c	Red	9.6
Lentil lectin (3 bands)	—	7.8, 8.0, 8.2
Human hemoglobin C	Red	7.5
Human hemoglobin A	Red	7.1
Equine myoglobin (2 bands)	Brown	7.0
Human carbonic anhydrase	—	6.5
Bovine carbonic anhydrase	—	6.0
β-Lactoglobulin B	—	5.1
Phycocyanin (3 bands)	Blue	4.45, 4.65, 4.75

\* Because the IEF standards are in native form, they cannot be used with reducing or denaturing agents such as urea, β-mercaptoethanol, or dithiothreitol. For calibration of IEF tube gels containing urea, use 2-D SDS-PAGE standards.



**IEF standards.**  
The gel was stained with Crocein Scarlet.

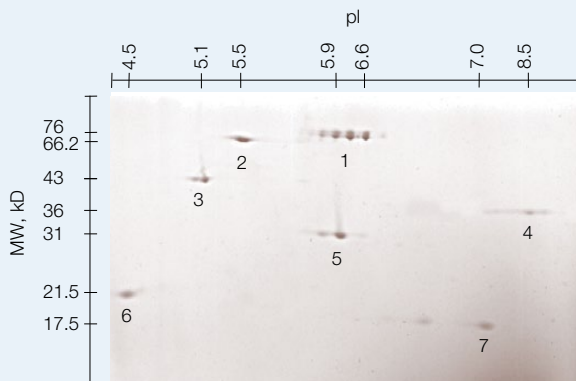
## Specialty Protein Standards for 2-D SDS-PAGE

### 2-D SDS-PAGE Standards

- Provide calibrated references for the pI and MW of proteins in 2-D SDS-PAGE applications
- Consist of 7 reduced, denatured proteins that can be visualized with silver or Coomassie Blue staining

### Constituent proteins of 2-D SDS-PAGE standards.

Protein	pI	MW (kD)
1. Hen egg white conalbumin	6.0, 6.3, 6.6	76
2. Bovine serum albumin (BSA)	5.4, 5.5, 5.6 (empirically determined)	66.2
3. Bovine muscle actin	5.0, 5.1 (empirically determined)	43
4. Rabbit muscle GAPDH	8.3, 8.5	36
5. Bovine carbonic anhydrase	5.9, 6.0	31
6. Soybean trypsin inhibitor	4.5	21.5
7. Equine myoglobin	7.0	17.5



**Two-dimensional electrophoretic protein pattern of 2-D SDS-PAGE standards.** The standards (2.5 μl) were run on 7 cm ReadyStrip™ IPG strips, then in the Mini-PROTEAN® II cell. For method details, see Klose (1975), Klose and Feller (1981), and Jungblut and Seifert (1990). The gel was stained with Bio-Rad's silver stain kit.

## References

Jungblut PR and Seifert R, Analysis by high-resolution two-dimensional electrophoresis of differentiation-dependent alterations in cytosolic protein pattern of HL-60 leukemic cells, *J Biochem Biophys Methods* 21, 47-58 (1990).

Klose J, Protein mapping by combined isoelectric focusing and electrophoresis of mouse tissues. A novel approach to testing for induced point mutations in mammals, *Humangenetik* 26, 231-243 (1975).

Klose J and Feller M, Two-dimensional electrophoresis of membrane and cytosol proteins of mouse liver and brain, *Electrophoresis* 2, 12-24 (1981).

## Ordering Information

Catalog # Description

### Recombinant Prestained Protein Standards

161-0393	<b>Precision Plus Protein All Blue Standards Value Pack</b> , 5 x 500 µl, 250 applications
161-0373	<b>Precision Plus Protein All Blue Standards</b> , 500 µl, 50 applications
161-0394	<b>Precision Plus Protein Dual Color Standards Value Pack</b> , 5 x 500 µl, 250 applications
161-0374	<b>Precision Plus Protein Dual Color Standards</b> , 500 µl, 50 applications
161-0397	<b>Precision Plus Protein Dual Xtra Standards Value Pack</b> , 5 x 500 µl, 250 applications
161-0377	<b>Precision Plus Protein Dual Xtra Standards</b> , 500 µl, 50 applications
161-0395	<b>Precision Plus Protein Kaleidoscope Standards Value Pack</b> , 5 x 500 µl, 250 applications
161-0375	<b>Precision Plus Protein Kaleidoscope Standards</b> , 500 µl, 50 applications
161-0399	<b>Precision Plus Protein WesternC Standards Value Pack</b> , 5 x 250 µl, 250 applications
161-0398	<b>Precision Plus WesternC (Standards + HRP) Value Pack</b> , 5 x 250 µl, 250 applications
161-0376	<b>Precision Plus Protein WesternC Standards</b> , 250 µl, 50 applications

### Recombinant Unstained Protein Standards

161-0396	<b>Precision Plus Protein Unstained Standards Value Pack</b> , 5 x 1000 µl, 500 applications
161-0363	<b>Precision Plus Protein Unstained Standards</b> , 1000 µl
161-0378	<b>Precision Plus Protein Unstained Standard Plugs</b> , 24 plugs

### Natural Prestained SDS-PAGE Protein Standards

161-0324	<b>Kaleidoscope Prestained Standards</b> , 500 µl
161-0305	<b>Prestained SDS-PAGE Standards</b> , low range, 500 µl
161-0309	<b>Prestained SDS-PAGE Standards</b> , high range, 500 µl
161-0318	<b>Prestained SDS-PAGE Standards</b> , broad range, 500 µl

### Natural Unstained SDS-PAGE Protein Standards

161-0303	<b>SDS-PAGE Standards</b> , high range, 200 µl
161-0304	<b>SDS-PAGE Standards</b> , low range, 200 µl
161-0317	<b>SDS-PAGE Standards</b> , broad range, 200 µl
161-0326	<b>Polypeptide SDS-PAGE Standards</b> , 200 µl

### Specialty Protein Standards

161-0310	<b>IEF Standards</b> , 250 µl
161-0320	<b>2-D Standards</b> , 250 µl

### Accessory Reagents

161-0380	<b>Precision Protein StrepTactin-HRP Conjugate</b> , 300 µl
161-0382	<b>Precision Protein StrepTactin-AP Conjugate</b> , 300 µl
170-6528	<b>Avidin-HRP</b> , 2 ml
170-6533	<b>Avidin-AP</b> , 1 ml
170-3554	<b>Streptavidin-AP</b> , 0.5 ml

### Premixed Sample Buffers

161-0737	<b>Laemmli Sample Buffer</b> , 30 ml
161-0738	<b>Native Sample Buffer</b> , 30 ml
161-0739	<b>Tricine Sample Buffer</b> , 30 ml
161-0768	<b>TBE-Urea Sample Buffer</b> , 30 ml
161-0763	<b>IEF Sample Buffer</b> , 30 ml
161-0764	<b>Zymogram Sample Buffer</b> , 30 ml
161-0767	<b>Nucleic Acid Sample Buffer</b> , 5x, 10 ml
161-0791	<b>XT Sample Buffer</b> , 4x, 10 ml

### Gel-Casting Buffers

161-0799	<b>Stacking Gel Buffer</b> , 0.5 M Tris-HCl, pH 6.8, 1 L
161-0798	<b>Resolving Gel Buffer</b> , 1.5 M Tris-HCl, pH 8.8, 1 L

### Premixed Electrophoresis Buffers

161-0732	<b>10x Tris/Glycine/SDS</b> , 1 L
161-0772	<b>10x Tris/Glycine/SDS</b> , 5 L cube
161-0734	<b>10x Tris/Glycine</b> , 1 L
161-0771	<b>10x Tris/Glycine</b> , 5 L cube
161-0744	<b>10x Tris/Tricine/SDS</b> , 1 L
161-0761	<b>10x IEF Anode Buffer</b> , 250 ml
161-0762	<b>10x IEF Cathode Buffer</b> , 250 ml
161-0733	<b>10x Tris/Boric Acid/EDTA (TBE)</b> , 1 L
161-0770	<b>10x Tris/Boric Acid/EDTA (TBE)</b> , 5 L cube
161-0741	<b>10x Tris/Boric Acid/EDTA (TBE)</b> , extended range, 1 L
161-0743	<b>50x Tris/Acetic Acid/EDTA (TAE)</b> , 1 L
161-0773	<b>50x Tris/Acetic Acid/EDTA (TAE)</b> , 5 L cube
161-0765	<b>10x Zymogram Renaturation Buffer</b> , 125 ml
161-0766	<b>10x Zymogram Development Buffer</b> , 125 ml

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