

# Featuring the Gold Standards in Band Identification





With Invitrogen's protein standards you can expect:

- Clear band resolution
- Accurate, easy-to-read
  results
- A time-saving, load-andgo format



## Setting the Standard

nvitrogen's complete line of protein markers sets the standard in electrophoresis band identification. Eight different protein standards are available, each with unique advantages, all offering maximum convenience. With every standard, you'll get:

- Unambiguous identification-proteins resolve into clear, sharp bands for precise results
- Wide size range of protein markers—enables you to identify diverse protein molecular weights easily
- Load-and-go format—standards are supplied ready to use, with no need to mix, reduce, or heat before using
- Consistent high quality—standards are strictly quality controlled to ensure consistent band intensity

Page

### Table of contents

### Description

	. age
Application Overview	3
Pre-Stained Molecular Weight Standards	4-7
MultiMark® Multi-Colored Standard	4
SeeBlue® Plus2 Pre-Stained Standard	5
SeeBlue® Pre-Stained Standard	6
BenchMark™ Pre-Stained Protein Ladder	7
Unstained Molecular Weight Standards	8-9
Mark12 <sup>™</sup> Unstained Standard	8
BenchMark™ Protein Ladder	9
MagicMark™ Western Protein Standard	10
SERVA® IEF 3-10 Marker	11
Ordering Information.	12

## A standard for every application

A broad selection of protein standards is available to meet your electrophoresis needs. Whether you're approximating molecular weight, verifying transfer efficiency, or determining protein isoelectric point, you're sure to find a standard that meets your application needs (Table 1). Each protein standard is supplied in a ready-to-use format, eliminating the need to dilute, mix, or heat before loading.

#### Molecular weight standards for SDS-PAGE

Protein molecular weight standards provide the means to estimate molecular weight as well as to confirm electrophoresis and transfer runs in SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis). By constructing a standard curve with a series of standards, you can estimate the apparent molecular weight of a sample protein based on its relative mobility. Unstained protein molecular weight standards (*i.e.*, Mark12<sup>™</sup> Unstained Standard, BenchMark<sup>™</sup> Protein Ladder) provide more accurate size estimation than pre-stained standards. Pre-stained standards, however, are well suited for confirming electrophoresis runs and estimating transfer efficiency. They also offer good molecular weight approximation if they are calibrated according to the gel/buffer system in use.

#### Marker for western blots

MagicMark<sup>™</sup> Western Protein Standard provides an easy and convenient means to accurately estimate protein molecular weight directly on western blots. You can visualize MagicMark<sup>™</sup> standard bands simultaneously with your target protein using the same antibody conjugate and protocol.

#### IEF marker for isoelectric focusing gel

The SERVA® Liquid Mix IEF Marker enables you to determine the isolectric point (pI) of unknown protein samples on horizontal and vertical isolectric focusing (IEF) gels.

Standard	MW/pl range	Advantage
MultiMark® Multi-Colored Standard	4-250 kDa $^{\dagger}$	Multi-colored bands for at-a-glance identification
SeeBlue® Plus2 Pre-Stained Standard	4-250 kDa <sup>†</sup>	Sharply resolved bands, including two colored bands for easy analysis
SeeBlue® Pre-Stained Standard	4-250 kDa $^{\dagger}$	Sharpest, most consistent pre-stained bands
BenchMark <sup>™</sup> Pre-Stained Protein Ladder	$\sim$ 10-190 kDa <sup>†</sup>	Sharp blue bands with one pink band for easy band identification
Mark12 <sup>™</sup> Unstained Standard	2.5-200 kDa	Accurate estimation of molecular weight with the broadest molecular weight range
BenchMark <sup>™</sup> Protein Ladder	10-220 kDa	Accurate estimation of molecular weight including two triple-intensity bands for easy reference
MagicMark <sup>™</sup> Western Protein Standard	20-120 kDa	Accurate molecular weight estimation directly on western blots
SERVA® Liquid Mix IEF Marker	pI 3.5-10.7	Accurate determination of protein isoelectric points

#### Table 1 - Advantages of Invitrogen's protein standards

<sup>†</sup> Actual range is dependent upon gel type and buffer system.



### For the easiest molecular weight band identification

The MultiMark<sup>®</sup> Multi-Colored Standard gives you a colorful alternative to blue, pre-stained molecular weight markers. It consists of 9 multi-colored bands (Figure 1).

Because each band is a different color, you'll be able to easily and immediately identify the molecular weight of each protein.

		SDS-PAGE System								
	Protein	Tris-Glycine	Tricine	NuPAGE <sup>®</sup> Bis-Tris (MES)	NuPAGE® Bis-Tris (MOPS)	NuPAGE <sup>®</sup> Tris Acetate				
	Myosin	250	208	185	188	209				
-	Phosphorylase B	148	105	98	97	111				
	Glutamic Dehydrogenase	60	53	52	52	52				
-	Carbonic Anhydrase	42	34	31	33	34				
	Myoglobin-Blue	30	23	19*	21*	n/a				
	Myoglobin-Red	22	17	17*	19*	n/a				
-	Lysozyme	17	13	11	12	n/a				
	Aprotinin	6	7	6	n/a	n/a				
	Insulin, B chain	4	4	3	n/a	n/a				

#### Figure 1 - Apparent molecular weights<sup>†</sup> of the MultiMark® Multi-Colored Standard

NuPAGE<sup>®</sup> 4-12% Bis-Tris Gel w/MES SDS Buffer Approximate Molecular Weights (kDa)

\* Note: The 2 migration patterns of Myoglobin Red and Blue are reversed in the NuPAGE® Bis-Tris MES and MOPS Buffers compared to the Tris-Glycine and Tricine Systems.

<sup>†</sup> Migration patterns in several buffer systems are shown because protein bands will have different mobilities in different SDS-PAGE buffer systems. For more information on this phenomenon, contact a Technical Service Representative at 800 955 6288, ext. 2 or review the technical note entitled "Accurate calibration of molecular weight standards for different buffer systems" on our web site at www.invitrogen.com.

### Sharp bands and easy analysis

The SeeBlue® Plus2 Pre-Stained Standard consists of 10 pre-stained protein markers–8 blue and 2 colored–that resolve into sharp distinct bands (Figure 2). The two colored bands make it easy to immediately identify the protein markers.

#### Figure 2 - Apparent molecular weights\* of the SeeBlue® Plus2 Pre-Stained Standard

	SDS-PAGE System						
Protein	Tris-Glycine	Tricine	NuPAGE <sup>®</sup> Bis-Tris (MES)	NuPAGE® Bis-Tris (MOPS)	NuPAGE® Tris-Acetate		
Myosin	250	210	188	191	210		
Phosphorylase B	148	105	98	97	111		
BSA	98	78	62	64	71		
Glutamic Dehydrogenase	64	55	49	51	55		
Alcohol Dehydrogenase	50	45	38	39	41		
Carbonic Anhydrase	36	34	28	28	n/a		
Myoglobin-Red	22	17	17	19	n/a		
Lysozyme	16	16	14	14	n/a		
Aprotinin	6	7	6	n/a	n/a		
Insulin, B chain	4	4	3	n/a	n/a		

NuPAGE<sup>®</sup> 4-12% Bis-Tris Gel w/MES SDS Buffer Approximate Molecular Weights (kDa)

\* Migration patterns in several buffer systems are shown because protein bands will have different mobilities in different SDS-PAGE buffer systems. For more information on this phenomenon, contact a Technical Service Representative at 800 955 6288, ext. 2 or review the technical note entitled "Accurate calibration of molecular weight standards for different buffer systems" on our web site at www.invitrogen.com.



### **Sharpest** band resolution

For sharp, consistent pre-stained bands, the SeeBlue<sup>®</sup> Pre-Stained Standard is the molecular weight standard of choice. It consists of 9 individual protein bands–all blue-that provide high resolution in any SDS-PAGE system (Figure 3). Since SeeBlue<sup>®</sup> is supplied pre-stained, there is no need to stain the gel in order to visualize the standard.

		SDS-PAGE System								
	Protein	Tris-Glycine	Tricine	NuPAGE <sup>®</sup> Bis-Tris (MES)	NuPAGE® Bis-Tris (MOPS)	NuPAGE <sup>®</sup> Tris Acetate				
	Myosin	250	210	188	191	210				
	BSA	98	78	62	64	71				
-	Glutamic Dehydrogenase	64	55	49	51	55				
-	Alcohol Dehydrogenase	50	45	38	39	41				
	Carbonic Anhydrase	36	34	28	28	n/a				
	Myoglobin	30	23	18	19	n/a				
	Lysozyme	16	16	14	14	n/a				
	Aprotinin	6	7	6	n/a	n/a				
	Insulin, B chain	4	4	3	n/a	n/a				

#### Figure 3 - Apparent molecular weights\* of the SeeBlue® Pre-Stained Standard

NuPAGE<sup>®</sup> 4-12% Bis-Tris Gel w/MES SDS Buffer Approximate Molecular Weights (kDa)

\* Migration patterns in several buffer systems are shown because protein bands will have different mobilities in different SDS-PAGE buffer systems. For more information on this phenomenon, contact a Technical Service Representative at 800 955 6288, ext. 2 or review the technical note entitled "Accurate calibration of molecular weight standards for different buffer systems" on our web site at www.invitrogen.com.

### Monitor electrophoretic separation in real time

The BenchMark<sup>™</sup> Pre-Stained Protein Ladder allows you to monitor the progress and quality of an electrophoretic separation. Like other pre-stained protein standards, you can also use the BenchMark<sup>™</sup> Pre-Stained Ladder to estimate the efficiency of protein transfer when performing western blotting. The standard proteins are affinity-purified and covalently coupled with dye. You'll see superb band sharpness (Figure 4), get easy orientation with a pink reference band, and approximate molecular weight without difficulty.



#### Figure 4 - BenchMark<sup>™</sup> Pre-Stained Protein Ladder

\* Coupling of the chromophores to the proteins affects their apparent molecular weight in SDS-PAGE relative to unstained standards. Each band in the pre-stained ladder is calibrated against unstained BenchMark\* Protein Ladder on a 4-20% Tris-Glycine gel and the apparent molecular weight is reported on the product profile. The pre-stained protein ladder should only be used to determine an approximate size molecular weight.



### Most accurate estimation of molecular weight

The 12 protein bands on the Mark12<sup>™</sup> Unstained Standard migrate the closest to their true molecular weight. That's because the dye used in pre-stained standards can affect band migration patterns, resulting in apparent molecular weights that are different from those of patterns in their unstained state. Since the proteins in the Mark12<sup>™</sup> stan-

dard are unstained, their migration pattern is not modified by the dye, allowing you to achieve the most accurate estimation of molecular weight. The Mark12<sup>™</sup> bands appear sharp and distinct when visualized with Coomassie<sup>®</sup> (Figure 5) or silver stain.

Figure 5 - The Mark 12<sup>™</sup> Unstained Standard

	Protein	Approximate Molecular Weights (kDa)
_	Myosin	200
	β-galactosidase	116.3
-	Phosphorylase B	97.4
_	Bovine serum albumin	66.3
	Glutamic dehydrogenase	55.4
_	Lactate dehydrogenase	36.5
	Carbonic anhydrase	31
-	Trypsin inhibitor	21.5
-	Lysozyme	14.4
-	Aprotinin	6
and the second	Insulin B chain	3.5
	Insulin A chain	2.5

NuPAGE® 4-12% Bis-Tris Gel w/MES stained with Coomassie® R-250

Note: The apparent molecular weights stated above apply to the Tris-glycine, Tricine, and NuPAGE® Systems.

### The benchmark of protein ladders

BenchMark<sup>™</sup> Protein Ladders are ideal for estimation of molecular weight of unknown proteins by SDSpolyacrylamide gel electrophoresis. Affinity-purified proteins generate sharp, intense bands without background for accuracy. You can visualize bands using either Coomassie<sup>®</sup> Brilliant Blue R-250 stain (Figure 6) or silver stain. Standard bands, including two tripleintensity reference bands, are in easy-to-identify increments for proper band identification.



#### Figure 6 - BenchMark<sup>™</sup> Protein Ladder



### Easy, accurate western blot analysis

MagicMark<sup>™</sup> Western Protein Standard lets you accurately estimate molecular weight directly on western blots. Each protein of the MagicMark<sup>™</sup> Standard contains an IgG binding site. You can visualize MagicMark<sup>™</sup> protein bands simultaneously with your target protein using the same antibody conjugate and protocol (Figure 7). Use chemiluminescent, fluorescent, or colorimetric detection methods for your analysis. With MagicMark<sup>™</sup>, you'll bypass steps required in conventional methods, yet obtain sharp bands and precise molecular weight estimation on your western blots.





MagicMark<sup>™</sup> Standard and an expressed protein containing a V5 epitope tag were separated on a NuPAGE<sup>®</sup> 4-12% Bis-Tris Gel and transferred to a nitrocellulose membrane. The blots were probed with a 1:5,000 dilution of mouse anti-V5 primary antibody and detected with the indicated western detection systems.

Lane 1: 5 µl of MagicMark<sup>™</sup> standard Lane 2: 2 ng of protein

For more information on MagicMark<sup>™</sup> Western Protein Standard, request the MagicMark<sup>™</sup> brochure at www.invitrogen.com.

### Accurate estimation of isolectric points

The SERVA<sup>®</sup> Liquid Mix IEF Marker provides 9 different proteins with 13 isoforms (Figure 8) for determining the isoelectric point (pl) of a full range of unknown protein samples in vertical or horizontal IEF gels. Since the standards are salt-free, you'll get straight bands for precise results. Unlike other IEF markers, the SERVA\* Marker contains bromophenol blue and methyl red dyes, so you can visualize the progress of the markers during electrophoresis.

#### Figure 8 - Schematic representation of SERVA® IEF markers in various pH fractions

Protein		Separated o	on pre-ca	ist SERV	ALYT P	RECOTI	ES* gel (f	lat bed)	separate	d on pro	e-cast ve	ertical ge	l (slab)
	191 1.1 (C)	pH 3-10	3-5	3-6	4-6	5-7	5-9	6-9	3-10	4-6	5-7	3-7	Cathode -
Cytochrome C	-	10.7					10.7	9.5	8.3		6.9	6.9	
Ribonuclease A	- 0	9.5	5.3	6.0	6.0	6:8			7,8	9.0			
isoform 1	70/	8.3					B.3	8.3	7.45			6.0	
isoform 2	7m/	7.8		den er			8.0		6.9	5.3			
Lectin	60	1174		5.3			7.6	8.0		5.2			
Myoglobin	64	6.9		5.2	5.3		<b>677</b>	7.8	6.0			5.3	
Carbonic anhydrase	5	6.0	4.5	1	5.2	6,0	6.9	7.4	5.3		6.0		
	1.1		4.2	4.5				6.9	4.5				
β-Lactoglobulin	40	53		4.0					4.2	4.5		4.5	
Trypsin inhibitor	_ 2	4.5	3.5	4,2	4.5	5.9	9,0		3.5	4.2	5.93	4.2	
Glucose oxidase	- 2	4.2			4.2	5.2	5.3	6.0			5.2		
Amyloglucosidase	=-1	3.5		3.5	3.5	4.5	4.5	5.3				3.5	Anode +

### A standard to meet your needs

At Invitrogen you're sure to find a standard for every protein electrophoresis application (Table 2). Each standard provides clear band resolution so you can accurately estimate molecular weights or isoelectric points. In addition, you'll save time with the suitable load-and-go format. Call and order today.

	MultiMark*	SeeBlue® Plus2/ SeeBlue®	BenchMark <sup>™</sup> Pre-stained	Mark 12™	BenchMark <sup>™</sup>	MagicMark <sup>∞</sup>	SERVA® IEF Marker 3-10
Application							
SDS-PAGE	Good	Good	Good	Good	Good	Good	n/a
IEF Gel	n/a	n/a	n/a	n/a	n/a	n/a	Best!
Western Blot	Good	Good	Good	Good	Good	Best!	n/a
Immediate Band Identification	Best!	Good	Good	*	*	*	*
Sharp Bands	Good	Best!	Good	Best!	Best!	Best!	Good
MW Estimation	Good	Good	Good	Best!	Best!	Best!	pI Estimation
Monitor Migration during Electrophoresi	Good	Good	Good	n/a	n/a	n/a	n/a
Silver Staining	Good	Good	Good	Best!	Best!	Good	Good
MW/pI Range	4-250 kDa	4-250 kDa	~ 10-190 kDa	2.5-200 kDa	10-220 kDa	20-120 kDa	pI 3.5-10.7
Туре	Natural Proteins	Natural Proteins	Recombinant Proteins	Natural Proteins	Recombinant Proteins	Recombinant Proteins	Natural Proteins

#### Table 2 - Choosing a protein standard

\* Bands visible only after staining.

Description	Quantity	Cat. no.
MultiMark <sup>®</sup> Multi-Colored Standard	500 µl	LC5725
SeeBlue® Plus2 Pre-Stained Standard	500 µl	LC5925
SeeBlue® Pre-Stained Standard	500 µl	LC5625
BenchMark <sup>™</sup> Pre-Stained Protein Ladder	2 x 250 µl	10748-010
Mark12 <sup>™</sup> Unstained Standard	1 ml	LC5677
BenchMark <sup>™</sup> Protein Ladder	2 x 250 µl	10747-012
MagicMark <sup>™</sup> Western Protein Standard	250 µl	LC5600
SERVA® IEF 3-10 Marker	500 µl	39212-01

BenchMark" Protein Ladder and BenchMark" Pre-stained Protein Ladder are covered by Limited Use Label License No. 41. Please refer to the Invitrogen web site or catalog for the corresponding Limited Use Label License statements. For research purposes only. Coomassie" is a registered trademark of Imperial Chemical Industries PLC.



Printed in the U.S.A. @2002 Invitrogen Corporation. Reproduction forbidden without permission.

#### Corporate headquarters:

1600 Faraday Avenue • Carlsbad, CA 92008 USA • Tel: 760 603 7200 • Fax: 760 602 6500 • Toll Free Tel: 800 955 6288 • E-mail: tech\_service@invitrogen.com • www.invitrogen.com • European headquarters:

Invitrogen Ltd • Inchinnan Business Park • 3 Fountain Drive • Paisley PA4 9RF, UK • Tel: +44 (0) 141 814 6100 • Fax: +44 (0) 141 814 6260 • E-mail: eurotech@invitrogen.com