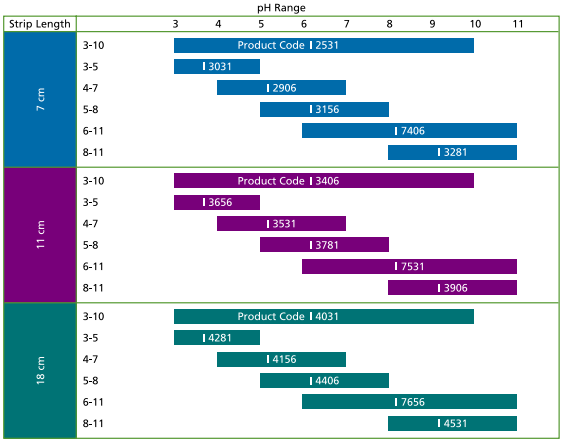
ELECTROPHORESIS

Two-Dimensional Electrophoresis

ProteoGel™ IPG Strips

Isoelectric focusing (IEF) represents the first dimension of two-dimensional (2D) electrophoresis, and immobilized pH gradient (IPG) strips facilitate this analysis. Each sample protein applied to an IPG strip will migrate to its isoelectric point (pI), the point at which its net charge is zero. ProteoGel IPG strips are available in three lengths to accommodate various gel sizes and six pH ranges to allow optimal separation.



Codes in colored bars represent Sigma product codes.
All IPG Strips are provided in packs of 12.

Features & Benefits

- **Narrow and wide range strips** with overlap options, in three lengths, allow optimal resolution of most protein samples
- **Control in manufacturing** ensures reproducible performance
- **IPG strips reduce preparation time** and reduce reagent waste
- **Strips are labeled for polarity** to ensure proper orientation

Manufactured by Proteome Systems™

Product Code	Description	Size
I 2531	7 cm, pH 3-10, ProteoGel IPG Strips	12 each
I 3031	7 cm, pH 3-5, ProteoGel IPG Strips	12 each
I 2906	7 cm, pH 4-7, ProteoGel IPG Strips	12 each
I 3156	7 cm, pH 5-8, ProteoGel IPG Strips	12 each
I 7406	7 cm, pH 6-11, ProteoGel IPG Strips	12 each
I 3281	7 cm, pH 8-11, ProteoGel IPG Strips	12 each
I 3406	11 cm, pH 3-10, ProteoGel IPG Strips	12 each
I 3656	11 cm, pH 3-5, ProteoGel IPG Strips	12 each
I 3531	11 cm, pH 4-7, ProteoGel IPG Strips	12 each
I 3781	11 cm, pH 5-8, ProteoGel IPG Strips	12 each
I 7531	11 cm, pH 6-11, ProteoGel IPG Strips	12 each
I 3906	11 cm, pH 8-11, ProteoGel IPG Strips	12 each
I 4031	18 cm, pH 3-10, ProteoGel IPG Strips	12 each
I 4281	18 cm, pH 3-5, ProteoGel IPG Strips	12 each
I 4156	18 cm, pH 4-7, ProteoGel IPG Strips	12 each
I 4406	18 cm, pH 5-8, ProteoGel IPG Strips	12 each
I 7656	18 cm, pH 6-11, ProteoGel IPG Strips	12 each
I 4531	18 cm, pH 8-11, ProteoGel IPG Strips	12 each

ProteoGel™ IPG Equilibration Buffer

ProteoGel IPG Equilibration Buffer has been specially formulated to simplify the transition between first and second dimensional analysis. Following isoelectric focusing, IPG strips must be equilibrated to further denature proteins, maintain solubility, and establish an appropriate pH for SDS-PAGE analysis. Convenient and practical, ProteoGel IPG Equilibration Buffer is compatible with all SDS-PAGE gel systems. Sold as bottles of powder that reconstitute with water to produce 50 ml of solution containing 0.05 M Tris-acetate, 2% SDS, 6 M urea, and 0.0067% bromophenol blue pH 7.0.

Product Code	Description	Size
I 7281	ProteoGel IPG Equilibration Buffer	1 bottle

Technology developed in partnership with Proteome Systems™

ELECTROPHORESIS

ProteoPrep™ Reduction and Alkylation Kit

The ProteoPrep™ Reduction and Alkylation Kit provides tributylphosphine (TBP) and iodoacetamide (IAA) for the reduction and alkylation of protein disulfide bonds. Procedures are provided for the reduction and alkylation of proteins in solution prior to loading the immobilized pH gradient (IPG) strip, during equilibration of the focused IPG strip prior to SDS-PAGE electrophoresis, or in-gel prior to tryptic digestion.

Features & Benefits

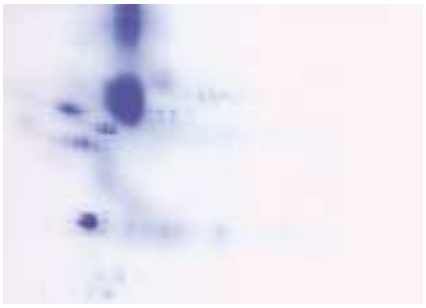
- **Reduction and alkylation of proteins** – *Reduces artifacts due to disulfide bond formation*
- **2D gels have less streaking, increased resolution, fewer artifacts** – *Better reproducibility when protein samples are reduced and alkylated prior to isoelectric focusing*

Components
Tributylphosphine Stock Solution
Alkylating Reagent Iodoacetamide

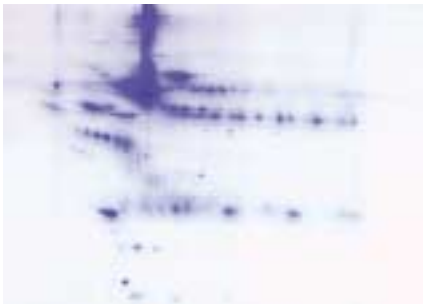
Product Code	Description	Size
PROT-RA	ProteoPrep Reduction and Alkylation Kit	1 kit

Sample/Gel conditions

Source, plasma, reduced; 1st dimension IEF 3-10, 2nd dimension 12% SDS-PAGE



Source, plasma, reduced and alkylated; 1st dimension IEF 3-10, 2nd dimension, 12% SDS-PAGE



*Herbert, B., Galvani, M., et al., Reduction and alkylation of proteins in preparation of two-dimensional map analysis: why, when and how?, Electrophoresis, **22,** 2046-2057 (2001).*

Above are examples of plasma in chaotropic membrane extraction reagent 2 (Product Code: C 0606), treated with and without alkylation reagent. Note the differences in resolution and clarity of the spots.

Additional Reduction and Alkylation Reagents

Product Code	Product Name	Application	References
A 4034	(+)-Sodium L-ascorbate (ascorbic acid)	for preparation of plant extracts; used as reductant for the isolation of chloroplasts	1. Gegenheimer, P., Methods Enzymol., **182,** 174-193 (1990).
D 8161	1,4-Dithioerythritol no protease detected (DTE)	reducing reagent; used for extracting membrane proteins and in preparing organelles due to its charge it may migrate out of IEF gels, affecting protein solubility	1. Pasquali, C., et al., Electrophoresis, **18,** 2573-2581 (1997). 2. Fialka, I., et al., Electrophoresis, **18,** 2582-2590 (1997).
D 9779	DL-Dithiothreitol no protease detected (DTT)	reducing agent; due to its charge it may migrate out of IEF gels, affecting protein solubility	1. Proteome Research: New Frontiers in Functional Genomics, Wilkins, M.R., Williams, K.L., Appel, R.D., Hochstrasser, D.F. (eds.) (Springer, 1997). 2. Jazwinski, S.M., Methods Enzymol., **182,** 154-174 (1990). 3. 2-D Proteome Analysis Protocols, Vol. **112,** Methods in Molecular Biology, Link, A.J. (ed.). (Humana Press, Totowa, NJ, 1999).
M 3148	2-Mercaptoethanol no protease detected (BME or 2-ME)	reducing agent; used at 10-140 mM for enhancing enzymatic lysis of yeast	1. Jazwinski, S.M., Methods Enzymol., **182,** 154-174 (1990).
T 7567	Tributylphosphine solution, 200 mM (0.5 ml per vial)	non-charged reducing reagent, keeps proteins soluble during electrophoresis	1. Herbert, B.R., Electrophoresis, **19,** 845-851 (1998). 2. Molloy, M.P., et al., **19,** 837-844 (1998). 3. Molloy, M.P., et al., Eur. J. Biochem., **267,** 2871-2881 (2000).
C 4706	Tris (2-carboxyethyl) phosphine HCl (TCEP)	water soluble reducing reagent; more stable than DTT and useful for mass spectrometry	1. Getz, et al., Anal Biochem., **273,** 73-80 (1999). 2. Fischer, W.H., et al., Rapid Commun. Mass Spectrom., **7,** 225-228 (1993).
A 3221	Iodoacetamide (56 mg per vial)	for alkylation of protein samples	1. Herbert, B.R., Electrophoresis, **19,** 845-851 (1998). 2. Moloy, M.P., et al., Electrophoresis, **19,** 837-844 (1998). 3. Molloy, M.P., et al., Eur. J. Biochem., **267,** 2871-2881 (2000).

ELECTROPHORESIS

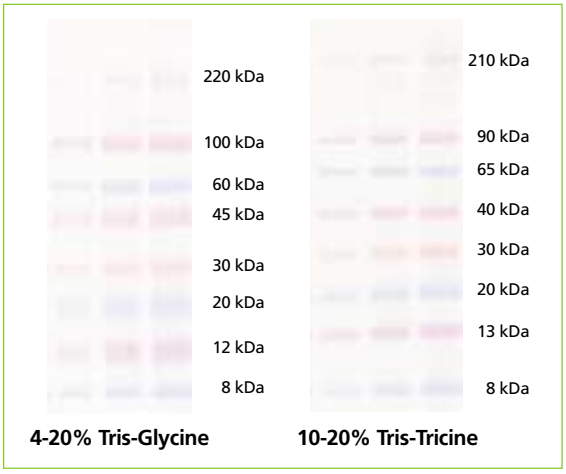
Protein molecular weight markers are used to calculate sample molecular weights, to monitor the progress of an electrophoretic run, or as a positive control for analysis conditions. Sigma offers a wide selection of markers for numerous protein electrophoresis applications, including silver staining, isoelectric focusing, fluorescent studies, and many more. All have been use-tested to assure outstanding performance and are available in convenient package sizes.

ColorBurst™ Marker

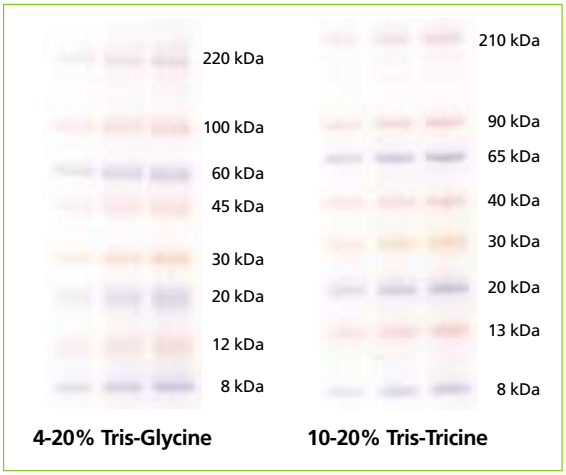
Brilliantly colored, exceptionally well resolved, convenient, and stable, ColorBurst protein molecular weight marker performs impressively in a variety of gel compositions and concentrations. ColorBurst is composed of eight proteins ranging from 8 to 220 kDa, which have been chemically reduced, alkylated, and conjugated to brilliantly colored dyes. ColorBurst can be used to estimate sample molecular weights, to monitor the progress of an electrophoretic run, or to confirm that an electroblot is complete. Each vial contains 500 µl of marker solution and may be used for up to 100 applications.

Features & Benefits

- **Exceptional resolution** allows estimation of sample molecular weights
- **No freeze/thaw cycle** means decreased degradation and long shelf life
- **Ready-to-use.** No boiling required before use
- **No chemical reduction** necessary prior to gel loading



Both gels were loaded (left to right) with 3, 5 and 7 µl of ColorBurst Marker. The marker was run using standard conditions on 10 x 10 cm, 1 mm thick, 10-well precast gels.



Bands transferred to nitrocellulose membranes from the gels in Figure 1. Transfers were completed in 90 minutes at 70 volts with Towbin's buffer (Tris-Glycine in 20% methanol.) Molecular Masses (kDa) given are apparent values as compared to Sigma's wide range marker set (M 4038). The Colorburst markers migrate differently with respect to M 4038 in the different gel systems tested.

Product Code	Description	Size
C 4105	ColorBurst Marker	1 vial

ELECTROPHORESIS

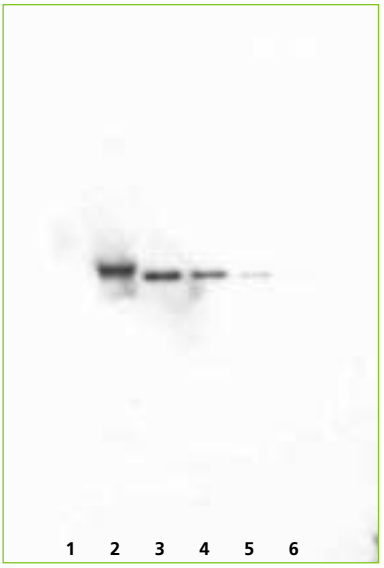
Chemichrome™ Western Control

Chemichrome Western Control is an ideal molecular weight marker and positive control for electrophoresis and subsequent Western blotting. Similar to ColorBurst™, Chemichrome contains an additional band of mouse IgG. During electrophoresis, brightly colored protein bands serve as positive controls for protein migration. During blotting, the same brightly colored bands indicate that the transfer is complete. After incubation with mouse primary and secondary antibodies, the band of mouse IgG (heavy chain) serves as a positive control using either colorimetric or chemiluminescent substrates. Simple to use and extremely practical, Chemichrome Western Control enables researchers to confirm the success of their analyses every step of the way. Chemichrome is supplied in vials containing 200 µl of ready-to-use solution.

Features & Benefits

- **Confirms** that a membrane transfer is complete
- **Confirms** successful Western Blotting conditions
- **Compatible** with many peroxidase and phosphatase substrates, including TMB and ECL®

Product Code	Description	Size
C 4236	Chemichrome Western Control	1 vial

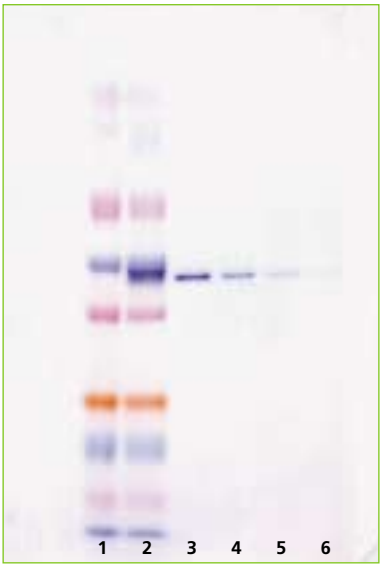


Colorburst (lane 1, undetected), Chemichrome (lane 2), and FLAG®-BAP concentrations of 80 ng, 40 ng, 20 ng and 10 ng (lanes 3-6 respectively) were run on a 6-15% Tris-Acetate gel, transferred to PVDF and developed with a chemiluminescent peroxidase substrate (CPS-1) for approximately 5 seconds.

Chemichrome™ Ultimate

Like Colorburst™ and Chemichrome™ Western Control, Chemichrome Ultimate contains eight brilliantly colored polypeptides which serve as positive controls during protein migration and transfer. In addition to mouse IgG, Chemichrome Ultimate also contains goat and rabbit IgG, which serve as positive controls for Western blotting using either colorimetric or chemiluminescent substrates.

Product Code	Description	Size
C 8738	Chemichrome Ultimate	1 vial



Colorburst (lane 1), Chemichrome (lane 2), and FLAG®-BAP concentrations of 80 ng, 40 ng, 20 ng and 10 ng (lanes 3-6 respectively) were run on a 6-15% Tris-Acetate gel, transferred to PVDF and developed with a TMB substrate (T 0565).

ProteoProfile™ PTM Marker

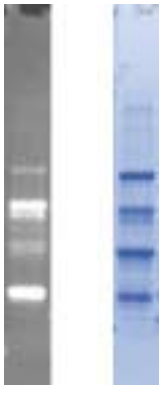
Glycosylation and phosphorylation are frequently encountered post-translation modifications in proteomic analysis. ProteoProfile PTM Marker, containing glycosylated and phosphorylated proteins, is designed for use as a positive and negative control in studying these post-translation modifications of proteins in SDS-PAGE. Electrophoresis will yield four protein bands:

BSA (not glycosylated, not phosphorylated)

Ovalbumin (glycosylated and phosphorylated)

β-Casein (not glycosylated, phosphorylated)

RNase B (glycosylated, not phosphorylated)



GlycoProfile III (PP0300) EZBlue™ (G 1041)

Superior Selectivity *ProteoProfile PTM Marker (P 1745), containing glycosylated and non-glycosylated proteins, was separated by electrophoresis on a 4-20% SDS-PAGE gel. The gel was stained with GlycoProfile III (PP0300) (left), fluorescently imaged, and then stained for total protein with EZBlue™ Gel staining reagent (G 1041) (right). Each band represents approximately 300 ng of protein.*

Product Code	Description	Size
P 1745	ProteoProfile PTM Marker	0.1 ml

ELECTROPHORESIS

Protein Molecular Weight Markers

Protein	Subunit MW (Da)	SigmaMarker™ MW Markers				Fluorescent MW Markers		Silver Stain MW Markers		
		M 3788	M 4038	M 3913	M 3546	F 3526	F 3401	M 5505	M 6539	M 5630
Urease, jack bean (hexamer)	545,000									
Urease, jack bean (trimer)	272,000									
Violet protein-dye conjugate	220,000									
Myosin, rabbit muscle	205,000	■	■			■				
α_2-Macroglobulin	180,000								■	
Recombinant marker protein	150,000									
β-Galactosidase, *E. coli*	116,000	■	■			■		■	■	
Albumin dimer, bovine serum	132,000									
Recombinant marker protein or Pink protein-dye conjugate	100,000									
Phosphorylase b, rabbit muscle	97,000	■	■					■	■	
Fructose-6-phosphate kinase	84,000	■	■							
Recombinant marker protein	75,000									
Albumin, bovine serum	66,000	■	■	■		■		■	■	■
Blue protein-dye conjugate	60,000									
IgG marker	59,500									
Catalase, bovine liver	58,100									
Glutamic dehydrogenase, bovine liver	55,000	■	■							
Recombinant marker protein	50,000									
Fumerase, porcine heart	48,500							■	■	■
Ovalbumin, chicken egg or Pink protein-dye conjugate	45,000	■	■	■						
Alcohol dehydrogenase	39,800					■	■			
Glyceraldehyde-3-phosphate dehydrogenase	36,000	■	■	■						
Recombinant marker protein	35,000									
Orange protein-dye conjugate or β-Casein	30,000									
Carbonic anhydrase	29,000		■	■		■	■	■	■	■
Triosephosphate isomerase	26,600				■					
Recombinant marker protein	25,000									
Trypsinogen, bovine pancreas	24,000		■	■						
Trypsin inhibitor, soybean or Blue protein-dye conjugate	20,100		■	■		■	■			
β-Lactoglobulin, bovine milk	18,400								■	■
Myoglobin from horse heart or RNase B	17,000				■					
Recombinant marker protein	15,000									
Lysozyme, egg white	14,300									
α-Lactalbumin, bovine milk	14,200		■	■	■		■		■	■
Pink protein-dye conjugate	12,000									
Blue protein-dye conjugate	8,000									
Aprotinin, bovine lung	6,500		■	■	■		■		■	
Insulin Chain B, oxidized, bovine	3,496				■					
Bradykinin	1,060				■					

ELECTROPHORESIS

Protein	Subunit MW (Da)	Biotinylated MW Markers		Recombinant MW Markers	Non-Denaturing MW Markers	Colorburst™	Chemichrome™	Chemichrome™ Ultimate	PTM Marker
		B 2787	SDS-6B	M 0671	MWND500	C 4105	C 4236	C 8738	P 1745
Urease, jack bean (hexamer)	545,000								
Urease, jack bean (trimer)	272,000								
Violet protein-dye conjugate	220,000								
Myosin, rabbit muscle	205,000								
α_2-Macroglobulin	180,000								
Recombinant marker protein	150,000								
β-Galactosidase, *E. coli*	116,000								
Albumin dimer, bovine serum	132,000								
Recombinant marker protein or Pink protein-dye conjugate	100,000								
Phosphorylase b, rabbit muscle	97,000								
Fructose-6-phosphate kinase	84,000								
Recombinant marker protein	75,000								
Albumin, bovine serum	66,000								
Blue protein-dye conjugate	60,000								
IgG marker	59,500								
Catalase, bovine liver	58,100								
Glutamic dehydrogenase, bovine liver	55,000								
Recombinant marker protein	50,000								
Fumerase, porcine heart	48,500								
Ovalbumin, chicken egg or Pink protein-dye conjugate	45,000								
Alcohol dehydrogenase	39,800								
Glyceraldehyde-3-phosphate dehydrogenase	36,000								
Recombinant marker protein	35,000								
Orange protein-dye conjugate or β-Casein	30,000								
Carbonic anhydrase	29,000								
Triosephosphate isomerase	26,600								
Recombinant marker protein	25,000								
Trypsinogen, bovine pancreas	24,000								
Trypsin inhibitor, soybean or Blue protein-dye conjugate	20,100								
β-Lactoglobulin, bovine milk	18,400								
Myoglobin from horse heart or RNase B	17,000								
Recombinant marker protein	15,000								
Lysozyme, egg white	14,300								
α-Lactalbumin, bovine milk	14,200								
Pink protein-dye conjugate	12,000								
Blue protein-dye conjugate	8,000								
Aprotinin, bovine lung	6,500								
Insulin Chain B, oxidized, bovine	3,496								
Bradykinin	1,060								

ELECTROPHORESIS

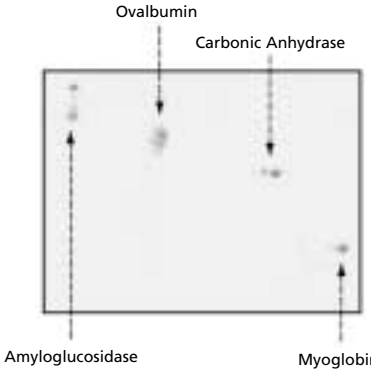
SDS-6B was separated on a 12.5% SDS-PAGE gel and transferred to PVDF (A) and nitrocellulose (B). The proteins were detected using Streptavidin-Peroxidase (S 5512) and 4-chloro-1-naphthol (C 8890).

Biotinylated Markers

Biotinylated molecular weight markers contain biotin-conjugated proteins which, following electrophoresis and membrane transfer, can be easily detected with a Streptavidin-Peroxidase Conjugate (S 5512) and a color development reagent such as TMB (T 0565).

Product Code	Description	MW Range (Da)
B 2787	Biotinylated SDS Molecular Weight Standard Mixture	Wide (6,500-180,000)
SDS-6B	Biotinylated SDS Molecular Weight Standard Mixture	Low (14,300-97,400)

These are both supplied in vials, each sufficient for 200 applications (on 10 x 10 cm mini-gels).



5 µl of 2D markers (M 3411) were separated with a mixture of ampholine pH 3.5-9.5 and ampholine pH 2.5-4.5 in the ratio 1:2.5 and then separated on a 12.5% SDS-PAGE gel. The gel was stained with Coomassie™ Brilliant Blue G (B 8522).

2D Electrophoresis Markers

Specifically designed for denaturing IEF and 2D electrophoresis, this molecular weight marker set contains four proteins selected to provide a diagonal pattern on a wide range 2D gel.

Product Code	Description	MW Range (Da)
M 3411	Marker for 2D Electrophoresis	17,000-89,000 7.6-3.8 pI

Sold in vials of approximately 200 µl of solution, sufficient for 20-40 applications.

Fluorescent Markers

Sigma fluorescent molecular weight markers are composed of FITC-conjugated proteins, which are well resolved and easily visualized with ultraviolet light in polyacrylamide gels or membranes.

Product Code	Description	MW Range (Da)
F 3526	Fluorescent Molecular Weight Marker	High (20,100-205,000)
F 3401	Fluorescent Molecular Weight Marker	Low (6,500-39,800)

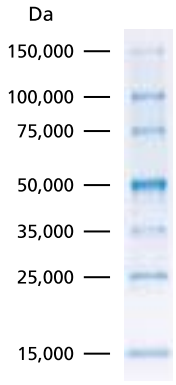
Sold in vials of powder that, when reconstituted with water, are sufficient for at least 25 applications (for 10 x 10 cm mini-gels).

ELECTROPHORESIS

Recombinant Markers

The Recombinant Molecular Weight Standard Mixture contains seven precisely sized proteins, molecular weights 15, 25, 35, 50, 75, 100, and 150 kDa. Recombinant proteins form sharp bands for accurate sample MW calculations.

Product Code	Description	MW Range (Da)
M 0671	Recombinant Molecular Weight Standard Mixture	15,000-150,000

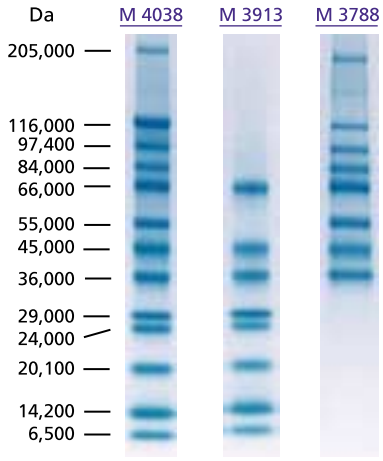


M 0671 (5 µl) was run on a 4-20% PAGE gel and stained with EZBlue™ (G 1041).

SigmaMarker™

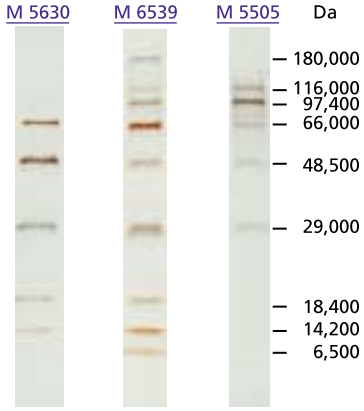
SigmaMarkers encompass the range of molecular weights common to most proteins and their subunits. Lyophilized with sample buffer, the wide, high, and low range markers are ready for use following reconstitution with water. They are formulated to yield a distribution of well-defined bands of approximately equal intensity following electrophoresis and Coomassie® Blue staining.

Product Code	Description	MW Range (Da)
M 4038	SigmaMarker	Wide (6,500-205,000)
M 3913	SigmaMarker	Low (6,500-66,000)
M 3788	SigmaMarker	High (36,000-205,000)



M 4038, M 3913, and M 3788 (5 µl) were run on a 4-20% PAGE gel and stained with EZBlue (G 1041).

ELECTROPHORESIS

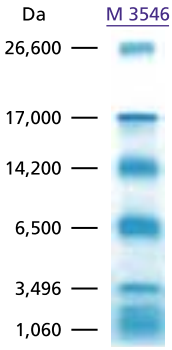


M 5630, M 6539, and M 5505 (7.5 µl of a 1:15 dilution) were run on a 10-18% SDS-PAGE gel and silver-stained.

Silver Stain Markers

Designed for molecular weight determinations on silver stained gels, Silver Stain SDS-PAGE molecular weight standard mixtures contain evenly distributed, well resolved proteins of equal intensity. **See also ProteoSilver™, page 110.**

Product Code	Description	MW Range (Da)
M 5630	Silver Stain SDS-PAGE Molecular Weight Standard	Low 14,000-66,000
M 6539	Silver Stain SDS-PAGE Molecular Weight Standard	Wide 6,500-180,000
M 5505	Silver Stain SDS-PAGE Molecular Weight Standard	High 29,000-116,000



M 3546 (5 µl of a 1:20 dilution) was run on a 10-20% Tris-Tricine SDS-PAGE gel, fixed with 5% glutaraldehyde and stained with EZBlue™ (G 1041).

Ultra-Low Range Markers

Sigma's Ultra-Low Range molecular weight marker is recommended for use with Tris-Tricine SDS-PAGE systems. Glutaraldehyde fixing is suggested to retain low molecular weight proteins.

Product Code	Description	MW Range (Da)
M 3546	Molecular Weight Marker for SDS-PAGE	Ultra-Low 1,000-26,600