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Unstained Protein Markers (Mark12™, HiMark™, BenchMark™)

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Protein Origins
Molecular Weights

Name	Product Description
Mark12™ Unstained Standard	The Mark12™ Unstained Standard is an unstained protein standard that allows the closest estimation of molecular weight of your sample protein over a wide molecular weight range. It is supplied ready-to-use for either Tris-Glycine, Tricine or NuPage Gels. Coomassie, silver or other protein staining methods are required to visualize this standard. Mark12™ has 12 bands with molecular weights ranging from 2.5 kDa to 200 kDa.
HiMark™ Unstained High Molecular Weight Protein Standard	The HiMark™ Unstained High Molecular Weight (HMW) Protein Standard allows accurate molecular weight estimation of high molecular weight proteins on Tris-Acetate Gels with Tris-Acetate SDS buffer system. HiMark™ Consists of 9 protein bands in the range of 40-500 kDa and is designed for use with NuPAGE® Novex 3-8% and 7% Tris-Acetate Gels under denaturing conditions. HiMark™ is Supplied in a ready-to-use format; do not heat or add reducing agent. Visualize HiMark™ using Coomassie, silver or other protein staining method.
BenchMark™ Protein Ladder	The BenchMark™ Protein Ladder consists of 15 engineered proteins ranging in molecular weight from 10 to 220 kDa. The proteins have been affinity purified for sharp bands and clear backgrounds, and are suitable for Coomassie or silver staining. The 20 and 50 kDa proteins are more prominent for easy orientation and to ensure proper identification of each protein. The ladder is in a ready-to-use format; do not heat or add reducing agent. This ladder can be used with Tris-Glycine, NuPAGE, or Tricine Gels.

Note: The HiMark™ Unstained HMW Protein Standard is designed for use with NuPAGE® Novex Tris-Acetate Gels under denaturing conditions. Using the standards with NuPAGE® Novex Bis-Tris Gels or Tris-Glycine Gels may result in inaccurate molecular weight estimation.

Protein Origins
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Mark12™

Protein	Origin
Myosin	Rabbit muscle
B-galactosidase	E. coli
Phosphorylase B	Rabbit muscle
Bovine serum albumin	Bovine serum
Glutamic dehydrogenase	Bovine liver
Lactate dehydrogenase	Porcine
Carbonic anhydrase	Bovine
Trypsin inhibitor	Soybean
Lysozyme	Egg white
Aprotinin	Bovine lung
Insulin B chain	Bovine pancreas
Insulin A chain	Bovine pancreas
Alcohol dehydrogenase	Baker's yeast
Myoglobin	Horse heart

BenchMark™ Standard

The ladder consists of a series of affinity purified, recombinant proteins. The exact protein make up of each band is proprietary.

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Mark12™ Unstained Standard

Protein	kDa
Myosin	200
B-Galactosidase	116.3
Phosphorylase b	97.4
BSA	66.3
Glutamic dehydrogenase	55.4
Lactate dehydrogenase	36.5
Carbonic anhydrase	31
Trypsin Inhibitor	21.5
Lysozyme	14.4
Aprotinin	6
Insulin B chain	3.5
Insulin A chain	2.5

HiMark™ Unstained High Molecular Weight Protein Standard

Band	kDa
1	500
2	290
3	240
4	160
5	116
6	97
7	66
8	55
9	40

HiMark™ Calculator - HiMark™ Calculator allows you to easily and accurately calculate the molecular weight of your proteins on NuPAGE® Novex 3-8% and 7% Tris-Acetate Gels and to extrapolate the molecular weight of proteins beyond the standard curve.
HiMark™ Calculator can be found on Invitrogen Website - http://www.invitrogen.com/content/sfs/manuals/HiMark™_Calculator_V1.xls.

BenchMark™ Protein Ladder

Band	kDa
1	220
2	160
3	120
4	100
5	90
6	80
7	70
8	60
9*	50
10	40
11	30
12	25
13*	20

14	15
15	10

Note: 50 & 20 kDa bands are darker intensity to serve as orientation bands.

Isoelectric Point
BenchMark™ Protein ladder:

Band	Isoelectric Point
220 kDa	6.87
160 kDa	6.74
120 kDa	6.61
100 kDa	6.53
90 kDa	4.45
80 kDa	4.46
70 kDa	6.37
60 kDa	4.49
50 kDa	4.53
40 kDa	6.24
30 kDa	4.64
25 kDa	4.98
20 kDa	5.56
15 kDa	5.51
10 kDa	5.42

SHIPPING CONDITIONS
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Name	Primary	Secondary
Mark12™ Unstained Standard	RT	Wet Ice
HiMark™ Unstained High Molecular Weight Protein Standard	Dry Ice	Dry Ice
BenchMark™ Protein Ladder	Dry Ice	Wet Ice

STORAGE CONDITIONS
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Name	Storage
Mark12™ Unstained Standard	+4°C
HiMark™ Unstained High Molecular Weight Protein Standard	-20°C (Avoid repeated freezing and thawing)
BenchMark™ Protein Ladder	-20°C (can also be stored at +4°C)

STABILITY
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Name	Stability
Mark12™ Unstained Standard	Guaranteed stable for 6 months when properly stored.
HiMark™ Unstained High Molecular Weight Protein Standard	Guaranteed stable for 6 months when properly stored.
BenchMark™ Protein Ladder	Guaranteed stable for 1 year when properly stored. [Stable 2.5 Years at -20°C, 4 months at 4°C, 1 month only at RT, 2 weeks only at 37°C. Stable for 50 freeze/thaws.]

QC SPECIFICATIONS
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Name	QC Specifications
Mark12™ Unstained Standard	*Gel Testing: 8% Tris-Glycine, 10−20% Tricine, and 10% Bis-Tris. *Twelve bands must be present on the 10−20% TR and the 10% BT. *Gel test performance must be comparable to 2 previously released lots. *Band intensity must not differ more than 20% from 2 previously released lots. *Migration: Bands must be straight and sharp. *Contaminant bands must be less than 20% of the intensity of the major bands.
HiMark™ Unstained High Molecular Weight Protein Standard	*Gel Testing: 3−8% and 7% Tris−Acetate gels. *Nine bands must be present on both gels. *Gel test performance must be comparable to 2 previously released lots. *Band intensity when stained must not differ more than 20% from 2 previously released lots. *Bands must be straight and sharp, and must migrate in distances comparable to the 2 previously released lots. *Contaminant bands must be less than 20% of the intensity of the major bands.
BenchMark™ Protein Ladder	*Fifteen major sharp bands must be present on a 4−20% Tris-Glycine gel. *Gel test performance must be comparable to a previously released lot. *Band intensity when stained with Coomassie Blue R−250 must not differ more than 20% from a previously released lot. The 20 kDa and the 50 kDa bands have greater intensity than the other bands. *Bands migrate comparable to a previously released lot *There is a faint contaminant band between the 80 and 90 kDa bands. All other contaminant bands must be less than 20% of the intensity of the major bands.

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General Notes
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- These protein standards are for SDS-PAGE and should not to be used for native electrophoresis. These markers are denatured and have SDS in the storage buffer.
- These standards are ready to load. There is no need to heat or add reducing agents. Do not boil - boiling may cause band degradation.
- Mark12^{TM} is modified to make re-oxidation impossible and to make this protein marker ready-to-use.
- Unstained markers like Mark12™, BenchMark™, and HiMark™ can be used for accurate molecular weight estimation of proteins. HiMark™ has a calculator to help in making a standard curve to estimate the molecular weight of an unknown protein. This calculator can be found on the Invitrogen website:
http://www.invitrogen.com/content/sfs/manuals/HiMark™_Calculator_V1.xls
- Pre-stained standards are ideal for monitoring an electrophoresis run, estimating the efficiency of transfer onto a membrane, and determining the *approximate* molecular weight of proteins. Note that the migration of a pre-stained standard is affected by factors such as pH and buffer system so in some cases the bands of a pre-stained standard may not match up exactly to the bands of an unstained standard. Note: Unstained standard is a more accurate determination of molecular weight.
- Blotting with HiMark™: To obtain higher transfer efficiency during the transfer of high molecular weight proteins, avoid using methanol in the transfer buffer. After transfer, you may stain the standard proteins on the membrane with Ponceau S, or any membrane stain of choice.

- Recommended transfer conditions: 1x NuPage transfer buffer; NO Methanol in the buffer; Add 0.1% SDS to the transfer buffer; 30V for 1 hour

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Quantitation/Determining Protein Concentration:
These markers are not designed for the quantitation and it is not recommended that these markers be used in the determination of protein concentration. Estimations of the protein concentration for Mark12™ are given below but these values are an approximation and should not be used to quantify samples.

Mark12™

Band	**per 5 µl**
Myosin	0.38 µg
B-Galactosidase	0.20 µg
Phosphorylase-b	0.35 µg
BSA	0.20 µg
GDH	0.60 µg
Lactate Dehydrogenase	0.40 µg
Carbonic Anhydrase	0.22 µg
Trypsin Inhibitor	0.32 µg
Lysozyme	0.25 µg
Aprotinin	0.38 µg
Insulin	1.12 µg (approx. 64% B, 35% A chain)

HiMark™ – No Quantitation Information

BenchMark™ Standard - Protein/Band is ~0.1 µg/µl

Troubleshooting
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Missing bands
- Check % gel that is being used. Depending on gel type and/or percentage, you may not see all the bands. For example, one would not see the smallest bands of the standard on a very low % gel. A high % gel may not resolve the higher MW bands.
- Check age of ladder – expired lots may see faded or missing bands.
- Check storage of ladder – adverse storage conditions will affect the stability of the ladder.
- Boiling of ladder may contribute to degradation/missing bands.

Smeary bands
- Don't load too much protein. See recommended load volumes in the manual.
- Bands will not be as well resolved on low % gels. Try using higher gel %.

Extra Bands
- Too much protein was loaded per lane. This is especially a problem with silver stained gels.
- Cross contamination in the lane from adjacent samples.

Marker has wrong MW on gel/blot
- Pre-Stained Standards show an apparent MW that is affected by pH of the gels and buffers used. Use Unstained standards for accurate molecular weight determination.
- Don't use these markers on a native gel. These markers are only for SDS-PAGE.
- For more information on factors affecting protein migration:
  Tung, Jwu-Sheng and Knight, C.A. (1972) *Analytical Biochemistry* 48, 153-163
  Matagne,A., Joris,B., Frere, J. (1991) *Biochem J.* 280, 553-556

Cross Reactivity on Western Blots

- See protein origins above in 'Components' section.

Poor Transfer

- Increase voltage, current or length of time for transfer.
- For transfer to PVDF, omit the SDS from the transfer buffer. SDS will cause the proteins to bind less efficiently to membranes because it disrupts the hydrophobic interaction between the membrane and the protein. If SDS is present in transfer buffer (i.e. to facilitate transfer of large proteins), make sure that there is no more than 0.02% SDS present in buffer.
- Methanol helps to enhance the hydrophobic "stick" of the proteins to the membrane. Too much methanol however, can be a problem as the proteins can become fixed in the gel. The methanol concentration in a western blot should be between 10 – 20%.

Marker goes through membrane during transfer

- Decrease voltage, transfer time.
- Ensure proper SDS, methanol concentration. Too much SDS can prevent binding to membrane. Alcohol enhances hydrophobic binding to membrane; not enough alcohol may prevent binding.
- Check pore size of membrane and size of proteins of interest. Proteins smaller than 10kDa will more easily transfer through a 0.45um pore size membrane. If smaller proteins are the target, 0.2um pore size will better capture those proteins smaller than 10kDa.

PRODUCT DOCUMENTATION

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Brochures **Citations** **Cell lines**

COA **FAQ** **Licensing**

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Vector Data

REFERENCES

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Factors affecting migration/apparent molecular weight

- Tung, Jwu-Sheng and Knight, C.A. (1972) *Analytical Biochemistry* 48, 153-163
- Matagne,A., Joris,B., Frere, J. (1991) *Biochem J*. 280, 553-556

PRODUCT NAME AND CATALOG NUMBERS

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Name	Size	Catalog Number
Mark12™ Unstained Standard	1 ml (200 applications of 5ul each)	LC5677
HiMark™ Unstained High Molecular Weight Protein Standard	250 µl (50 applications of 5 µl each)	LC5688
BenchMark™ Protein Ladder	2 x 250 µl (100 applications of 5 µl each)	10747012

COMPONENTS

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Name	Components
Mark12™ Unstained Standard	1 ml supplied in loading buffer containing Tris-HCl, Glycerol, SDS, Phenol red, Coomassie Blue G-250.
HiMark™ Unstained High Molecular Weight Protein Standard	250 mM Tris-HCl, pH 8.5; 0.5 mM EDTA; 50 mM DTT; 10% glycerol; 2% LDS; 0.2 mM Coomassie® G-250; 0.175 mM

	Phenol red.
BenchMark™ Protein Ladder	Two vials of 250 µl each are provided in loading buffer consisting of 50 mM Tris-HCl (pH 6.8); 2 mM EDTA; 10 mM DTT; 2% (w/v) SDS; 10% (w/v) Glycerol; 0.01%(w/v) Bromophenol blue.

ASSOCIATED PRODUCTS
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Novex Tris-Glycine Gels
NuPAGE Bis-Tris Gels
NuPAGE Tris-Acetate Gels

Need more help? Please email us by clicking here.