

Invitromass™ High Molecular Weight Mass Calibrant Kit

Cat. no. MC10002

Store at -20°C

Contents and Storage

The Invitromass™ High Molecular Weight (HMW) Mass Calibrant Kit contents are listed in the table below.

The Invitromass™ HMW Mass Calibrant Kit is shipped on dry ice. Upon receipt, store the contents at -20°C. The product is guaranteed stable for 6 months when stored properly. To avoid repeated freezing and thawing, aliquot calibrants in small volumes (5 µl) and store at -20°C.

Sufficient calibrants are supplied to prepare 100 x 1 µl spots.

Contents	Concentration	Quantity
Invitromass™ 30 kDa Mass Calibrant	0.3 µg/µl in 0.05% TFA	20 µl
Invitromass™ 50 kDa Mass Calibrant	0.5 µg/µl in 0.05% TFA	20 µl
Invitromass™ 70 kDa Mass Calibrant	1.05 µg/µl in 0.05% TFA	20 µl
Invitromass™ 90 kDa Mass Calibrant	1.8 µg/µl in 0.05% TFA	20 µl
Invitromass™ 160 kDa Mass Calibrant	0.48 µg/µl in 0.05% TFA	100 µl
Trifluoroacetic acid (TFA)	0.1%	2 x 1 ml
Acetonitrile	100%	2 x 1 ml
Sinapinic acid (Fluka cat. no. 85429)	Solid	2 x 20 mg
Matrix Buffer	Solvent	0.5 ml

Description

The Invitromass™ High Molecular Weight (HMW) Mass Calibrant Kit allows accurate mass calibration of intact proteins by MALDI-TOF (Matrix Assisted Laser Desorption/Ionization-Time Of Flight) MS (Mass Spectrometry) analysis. The Invitromass™ HMW Calibrants are formulated to provide strong ion signals when co-crystallized with an appropriate MALDI matrix. The accurate mass and absolute amount of each calibrant is known, allowing the optimization for accurate internal calibration.

Important features of the Invitromass™ HMW Calibrants are listed below:

- Consists of 5 purified, **recombinant** proteins that provide calibration in the range of 15,000-320,000 Da (the use of recombinant proteins as calibrants provides accurate mass determination as recombinant proteins are devoid of sequence heterogeneity associated with naturally occurring proteins)
- Specifically designed for tuning, calibration, and sensitivity testing of MALDI-TOF mass spectrometers
- Supplied in a ready-to-use format
- Optimized to produce intense singly charged ion $[M+H]^+$ while the presence of doubly charged ion $[M+2H]^{2+}$ and dimer $[2M+H]^+$ peaks for each protein provides a wider mass range for calibration
- Suitable for use as internal and external standards

Calibrating MALDI TOF Instrument with Calibrants

To calibrate a MALDI TOF mass spectrometer, the flight times of two ions with known masses are required. In a MALDI TOF instrument, the time of flight of an ion, t , is proportional to the square root of mass, m as shown in the equation: $t = am^{1/2} + b$. The flight times, t_1 and t_2 , of two known ions of mass, m_1 and m_2 , are used to calculate the parameters a and b . For accurate mass calibration, the m_1 and m_2 peaks must closely bracket the peak of interest indicating that mass calibrants with more than two calibration peaks can cover a wide mass range.

Each Invitromass™ HMW Calibrant consists of a purified recombinant protein that produces intense singly charged ion $[M+H]^+$, as well as shows the presence of doubly charged ion $[M+2H]^{2+}$ and dimer $[2M+H]^+$ peaks on MALDI-TOF MS analysis allowing the mass determination for unknown samples.

Note

The Invitromass™ HMW Mass Calibrants are designed for MALDI-TOF MS analysis. The MALDI matrix supplied with the kit allows you to get started with your analysis. To order more of the matrix, contact the manufacturer with catalog number listed in the above table.

Part No. MC10002.pps

Rev. Date: 14 June 2004

This product is distributed for laboratory research use only. CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

For technical questions about this product, call the Invitrogen TECH-LINE™ 800 955 6288

General Guidelines

- The Invitromass™ HMW Mass Calibrant Kit contains solvents; always wear a laboratory coat, disposable gloves, and eye protection when handling this kit
- To obtain the best results, always use reagents stored at the recommended temperature and prepare samples as described below
- Always use polypropylene tubes to minimize contaminations from ions such as Na, K ions and/or loss of peptides and protein
- After spotting Invitromass™ Calibrants on the MALDI plate, perform MALDI-TOF MS analysis within the same day

Preparing MALDI Matrix

The kit includes sinapinic acid matrix that is ideal for intact protein analysis.

1. To a clean microcentrifuge tube, add 700 μ l 100% acetonitrile and 700 μ l 0.1% TFA supplied in the kit. Mix well.
2. Add 700 μ l acetonitrile/TFA mixture from Step 1 to 20 mg sinapinic acid vial supplied in the kit.
3. Mix well by vortexing for 1 minute and centrifuge for 10-15 seconds.
4. Use the supernatant (saturated matrix solution) to prepare calibrants (see below).

If desired, store matrix solution at 4°C. The solution is stable at 4°C for 2 weeks.

Using 30, 50, 70, and 90 kDa Mass Calibrants

1. Thaw the appropriate calibrant at room temperature. Vortex the calibrant for 30 seconds.
2. If the sinapinic acid matrix solution prepared in acetonitrile/TFA mixture (see above) is stored at 4°C, allow the matrix to equilibrate to room temperature. Vortex for 1 minute to mix well.
3. In a clean microcentrifuge tube, mix 1 μ l calibrant with 4 μ l sinapinic acid matrix solution from Step 2. Vortex for 10-15 seconds. This provides sufficient calibrant solution for 5 spots and each spot contains 2 pmoles of purified, recombinant protein.
4. Spot 1 μ l calibrant/matrix solution on a MALDI plate. Allow the spot to air-dry (~ 5 minutes).
5. Spot 1 μ l protein sample in matrix solution on an adjacent well on the MALDI plate. Allow the spot to air dry (~ 5 minutes).
6. Perform calibration of the MALDI TOF MS instrument using linear mode. The average mass for each calibrant is listed below and spectra for each calibrant are shown on the following pages.

Using 160 kDa Mass Calibrant

1. Thaw the 160 kDa calibrant at room temperature. Vortex the calibrant for 30 seconds.
2. If the sinapinic acid matrix solution prepared in acetonitrile/TFA mixture (see above) is stored at 4°C, allow the matrix to equilibrate to room temperature. Vortex for 1 minute to mix well.
3. In a clean microcentrifuge tube, mix 4 μ l sinapinic acid matrix solution from Step 2 with 1 μ l Matrix Buffer supplied in the kit. Vortex for 15-20 seconds.
4. On a MALDI plate, mix 1 μ l calibrant with 1 μ l matrix/matrix buffer mixture from Step 3. Allow the spot to air-dry (~5 minutes). Each spot contains 3 pmoles of purified, recombinant protein.
5. Spot 0.5-1 μ l protein sample in matrix solution on an adjacent well on the MALDI plate. Allow the spot to air dry (~5 minutes).
6. Perform calibration of the MALDI TOF instrument using linear mode. The average mass for 160 kDa calibrant is listed below and spectrum for 160 kDa calibrant is shown on page 4.

Expected Average Mass of Invitromass™ HMW Calibrants

The expected average mass for each calibrant is listed in the table below.

Invitromass™ Calibrants	Average Mass					
	[M+H] ⁺	[2M+H] ⁺	[M+2H] ²⁺	[3M+H] ⁺	[3M+2H] ²⁺	[4M+H] ⁺
30 kDa	29,852 Da	59,703 Da	14,927 Da	89,554 Da	44,778 Da	119,405 Da
50 kDa	49,825 Da	99,649 Da	24,913 Da	149,473 Da	74,737 Da	199,297 Da
70 kDa	69,205 Da	138,409 Da	34,603 Da	207,613 Da	103,807 Da	276,817 Da
90 kDa	89,713 Da	179,425 Da	44,857 Da	269,137 Da	134,569 Da	--
160 kDa	159,081 Da	318,161 Da	79,541 Da	477,241 Da	238,621 Da	--

Troubleshooting

Review the information below to troubleshoot your experiments with Invitromass™ HMW Mass Calibrants.

No signal or low signal intensity is mainly due to improper sample preparation or use of improperly stored reagents

- Prepare the Invitromass™ Calibrants as described on the previous page and load at least 2-3 pmoles per spot to obtain a good signal. Always mix reagents by vortexing for best results. Be sure to add Matrix Buffer solution for the analysis of 160 kDa Calibrant. Avoid using discolored matrix.
- Make sure the Invitromass™ Calibrants and matrix solution are stored at the recommended temperature. Avoid using matrix solution that is stored at 4°C for more than 2 weeks or Invitromass™ Calibrants stored at room temperature.
- If you are using calibrants as internal standards, you may need to optimize the ratio of Invitromass™ Calibrant relative to your protein sample to obtain comparable signal intensity and accurate calibration.
- If poor peak shape is observed when calibrants are used as internal standards, use the calibrants as external standards.

High Background is due to the presence of contaminants

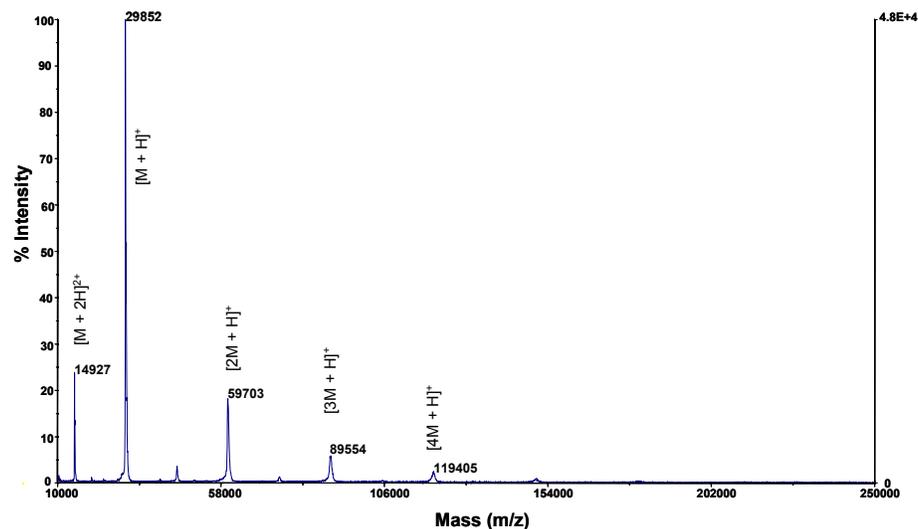
- Always use high-quality, ultra pure reagents for MALDI-TOF MS analysis
- Use clean MALDI plates for analysis and always use polypropylene tubes

Expected MALDI-TOF Spectra for Invitromass™ Calibrants

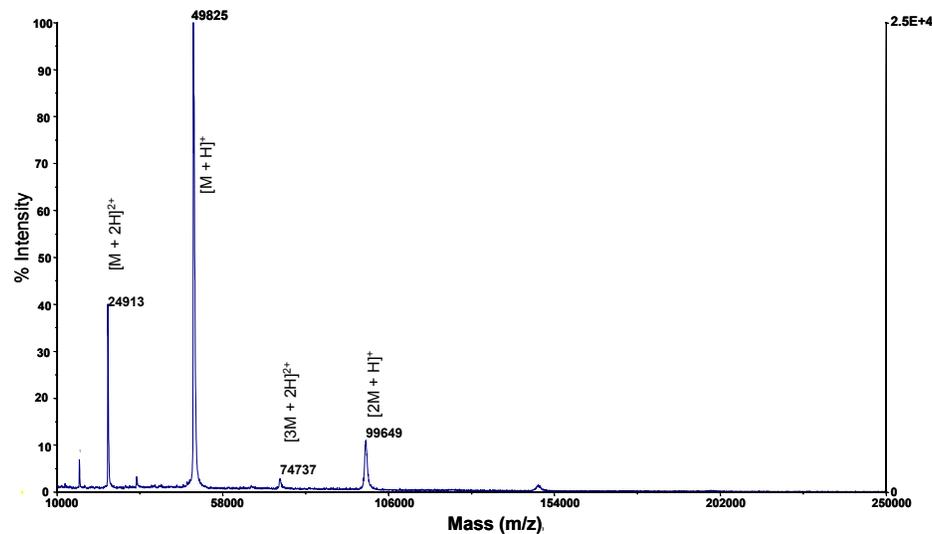
Invitromass™ Calibrants (2-3 pmoles) were analyzed by MALDI-TOF MS as described in this manual. The spectra shown below are obtained using a Voyager-DE™ STR MALDI TOF instrument in linear mode.

Note: The Invitromass™ Calibrant spectra may vary depending on the MALDI instrument and mode of analysis.

Invitromass™ 30 kDa Calibrant

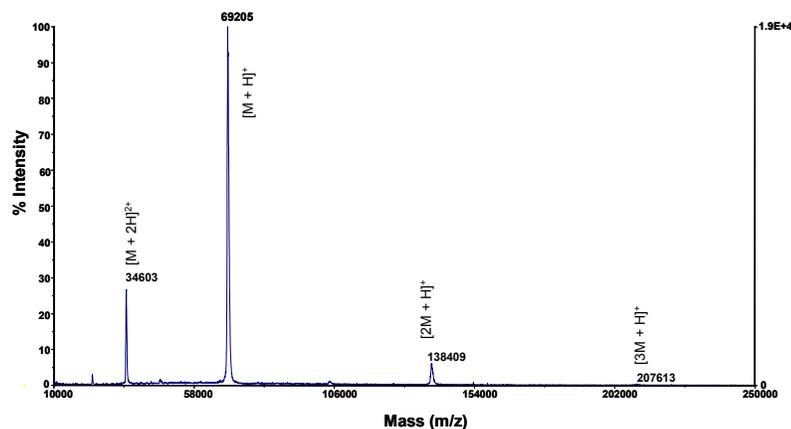


Invitromass™ 50 kDa Calibrant

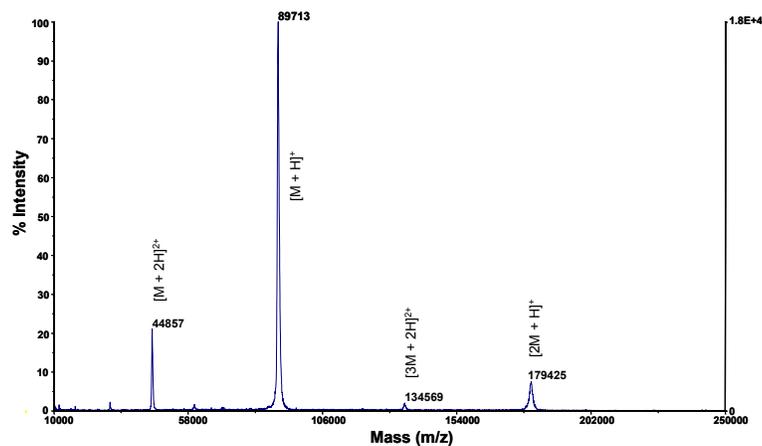


Expected MALDI-TOF Spectra for Invitromass™ Calibrants, Continued

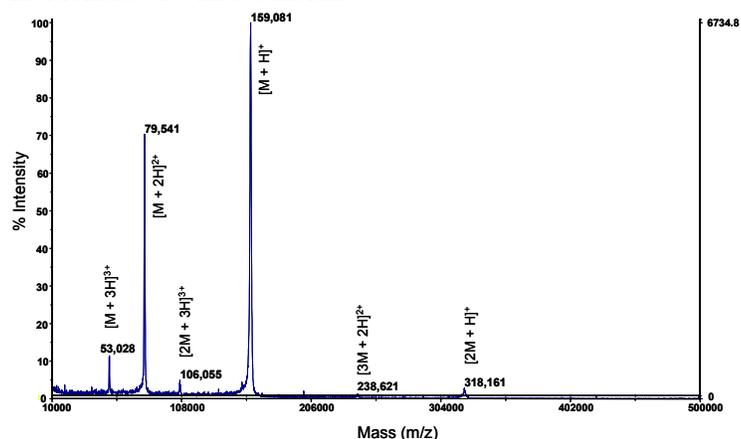
Invitromass™ 70 kDa Calibrant



Invitromass™ 90 kDa Calibrant



Invitromass™ 160 kDa Calibrant



Product Qualification

The Invitromass™ HMW Mass Calibrant Kit is qualified by direct MALDI-TOF MS analysis as described in this manual. The MALDI-MS analysis must yield the expected spectra for each calibrant and be devoid of any contaminant ions.

Limited Use Label License No. 201: Invitromass™ Molecular Weight MALDI-MS Calibrants

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