

# Invitromass™ Low Molecular Weight Mass Calibrant Kit

Cat. no. MC10001

Store at -20°C

## Contents and Storage

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

The Invitromass™ LMW Mass Calibrant Kit is shipped on dry ice. Upon receipt, store the contents at 4°C (short-term) or -20°C (long-term). The product is guaranteed stable for 6 months when stored at -20°C.

Sufficient calibrants are supplied to prepare 1125 x 1 µl spots.

Contents	Concentration	Quantity
Invitromass™ Calibrant 1 (500–1000 Da)	Bradykinin Fragment (1-5); 15.15 pmole/µl in 0.01% TFA Bradykinin Fragment (1-7); 16.67 pmole/µl in 0.01% TFA Lys(-Des-Arg <sup>9</sup> , Leu <sup>8</sup> )-Bradykinin; 18.18 pmole/µl in 0.01% TFA	45 µl
Invitromass™ Calibrant 2 (1000-3000 Da)	Lys(-Des-Arg <sup>9</sup> , Leu <sup>8</sup> )-Bradykinin; 22.22 pmole/µl in 0.01% TFA ACTH (1-16); 11.11 pmole/µl in 0.01% TFA ACTH (1-24); 16.67 pmole/µl in 0.01% TFA	45 µl
Invitromass™ Calibrant 3 (3000-6000 Da)	ACTH (1-24); 6.74 pmole/µl in 0.01% TFA ACTH (1-39); 15.17 pmole/µl in 0.01% TFA Insulin; 28.10 pmole/µl in 0.01% TFA	45 µl
Invitromass™ Calibrant 4 (6000-12000 Da)	Insulin; 4.17 pmole/µl in 0.01% TFA Ubiquitin; 25 pmole/µl in 0.01% TFA Cytochrome C; 20.83 pmole/µl in 0.01% TFA	40 µl
Trifluoroacetic acid (TFA)	0.1%	2 x 1 ml
Acetonitrile	100%	2 x 1 ml
Sinapinic acid (Fluka cat. no. 85429)	Solid	20 mg
α-cyano-4-hydroxycinnamic acid (Sigma cat. no. C2020)	Solid	3 x 20 mg

## Description

The Invitromass™ Low Molecular Weight (LMW) Mass Calibrant Kit allows accurate mass calibration of peptides, proteins, and organic molecules by MALDI-TOF (Matrix Assisted Laser Desorption/Ionization-Time Of Flight) MS (Mass Spectrometry) analysis. The Invitromass™ LMW 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. The Invitromass™ Calibrant 2 is specifically suited for peptide mass fingerprinting.

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

- Consists of 4 sets of calibrants for calibration of mass spectra (500-12,000 Da) and each set of calibrant contains a mixture of 3 peptides or proteins
- Designed for tuning, calibration, sensitivity testing, and performing operational qualification of linear and reflectron MALDI-TOF mass spectrometers
- Supplied in a ready-to-use format
- Optimized to produce intense singly charged ion  $[M+H]^+$  with comparable intensities while the presence of doubly charged ion  $[M+2H]^{2+}$  for each peptide or protein provides a wider mass range for calibration
- Suitable for use as internal and external standards

## Note

The Invitromass™ LMW 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 matrix, contact the manufacturer with catalog number listed on the above table.

Part No. MC10001.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<sup>SM</sup> 800 955 6288

## 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 suggesting that mass calibrants with more than two calibration peaks can cover a wide mass range. Each set of Invitromass™ LMW Calibrant produces 3 intense calibration peaks on MALDI-TOF MS analysis that allows mass determination of unknown samples.

### General Guidelines

- The Invitromass™ LMW 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
- Use  $\alpha$ -cyano-4-hydroxycinnamic acid matrix for Invitromass™ Calibrants 1, 2, and 3, and sinapinic acid matrix for Calibrant 4
- 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

1. To a clean microcentrifuge tube, add 500  $\mu$ l 100% acetonitrile and 500  $\mu$ l 0.1% TFA supplied in the kit. Mix well.
2. Add 500  $\mu$ l acetonitrile/TFA mixture from Step 1 to 20 mg sinapinic acid vial or to 20 mg  $\alpha$ -cyano-4-hydroxycinnamic acid matrix 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).
5. Store matrix solution at 4°C. The sinapinic acid matrix solution is stable at 4°C for 1 week and  $\alpha$ -cyano-4-hydroxycinnamic acid matrix solution is stable at 4°C for 2 weeks.

### Using Invitromass™ LMW Calibrants

1. Thaw the appropriate calibrant at room temperature. Vortex the calibrant for 30 seconds.
2. If the 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  $\mu$ l calibrant with 24  $\mu$ l appropriate matrix solution from Step 2 (use  $\alpha$ -cyano-4-hydroxycinnamic acid matrix for Invitromass™ Calibrants 1, 2, and 3, and sinapinic acid matrix for Calibrant 4). Vortex for 10-15 seconds. This provides sufficient calibrant solution for 25 spots. Be sure to use the calibrant/mixture solution within the same day to obtain the best results and maintain the solution at 4°C.
4. Spot 1  $\mu$ l calibrant/matrix solution on a MALDI plate. Allow the spot to air-dry (~ 5 minutes).
5. Spot 0.5-1  $\mu$ l peptide or protein sample in matrix solution on an adjacent well on the MALDI plate. Allow the spot to air dry (~5 minutes). You may co-spot the peptide or protein sample on the same spot as the calibrant, if desired.
6. Perform calibration of the MALDI-TOF MS instrument using linear or reflectron mode. The average and monoisotopic mass for each calibrant is listed below and spectra for each calibrant are shown on the following pages.

### Expected Average Mass of Invitromass™ LMW Calibrants

The expected average and monoisotopic molecular mass of the singly-charged molecular ion for each calibrant and formula for the unprotonated peptide or protein is listed below.

Invitromass™ Calibrants	Components	Average Mass [M+H] <sup>+</sup>	Monoisotopic Mass [M+H] <sup>+</sup>	Formula
Calibrant 1	Bradykinin Fragment (1-5)	573.67 Da	573.3148 Da	C <sub>27</sub> H <sub>40</sub> N <sub>8</sub> O <sub>6</sub>
	Bradykinin Fragment (1-7)	757.87 Da	757.3996 Da	C <sub>35</sub> H <sub>52</sub> N <sub>10</sub> O <sub>9</sub>
	Lys(-Des-Arg <sup>9</sup> , Leu <sup>8</sup> )-Bradykinin	999.20 Da	998.5786 Da	C <sub>47</sub> H <sub>75</sub> N <sub>13</sub> O <sub>11</sub>
Calibrant 2	Lys(-Des-Arg <sup>9</sup> , Leu <sup>8</sup> )-Bradykinin	999.20 Da	998.5786 Da	C <sub>47</sub> H <sub>75</sub> N <sub>13</sub> O <sub>11</sub>
	ACTH (1-16)	1938.26 Da	1936.9851 Da	C <sub>89</sub> H <sub>133</sub> N <sub>25</sub> O <sub>22</sub> S
	ACTH (1-24)	2934.49 Da	2932.5883 Da	C <sub>136</sub> H <sub>210</sub> N <sub>40</sub> O <sub>31</sub> S
Calibrant 3	ACTH (1-24)	2934.49 Da	2932.5883 Da	C <sub>136</sub> H <sub>210</sub> N <sub>40</sub> O <sub>31</sub> S
	ACTH (1-39)	4542.14 Da	4539.2670 Da	C <sub>207</sub> H <sub>308</sub> N <sub>56</sub> O <sub>58</sub> S
	Insulin	5734.60 Da	5730.6087 Da	C <sub>254</sub> H <sub>378</sub> N <sub>65</sub> O <sub>75</sub> S <sub>6</sub>
Calibrant 4	Insulin	5734.60 Da	5730.6087 Da	C <sub>254</sub> H <sub>378</sub> N <sub>65</sub> O <sub>75</sub> S <sub>6</sub>
	Ubiquitin	8565.80 Da	8560.6250 Da	C <sub>378</sub> H <sub>630</sub> N <sub>105</sub> O <sub>118</sub> S
	Cytochrome C	12,361.20 Da	--	C <sub>560</sub> H <sub>874</sub> N <sub>148</sub> O <sub>156</sub> S <sub>4</sub> Fe

## Troubleshooting

Review the information below to troubleshoot your experiments with Invitromass™ LMW 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 spot at least 0.5-1 µl per spot to obtain a good signal. Always mix reagents by vortexing for best results. Be sure to use the appropriate matrix solution calibrants (see previous page). 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™ LMW Calibrants

The Invitromass™ LMW Calibrants (0.5-1 µl) 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.

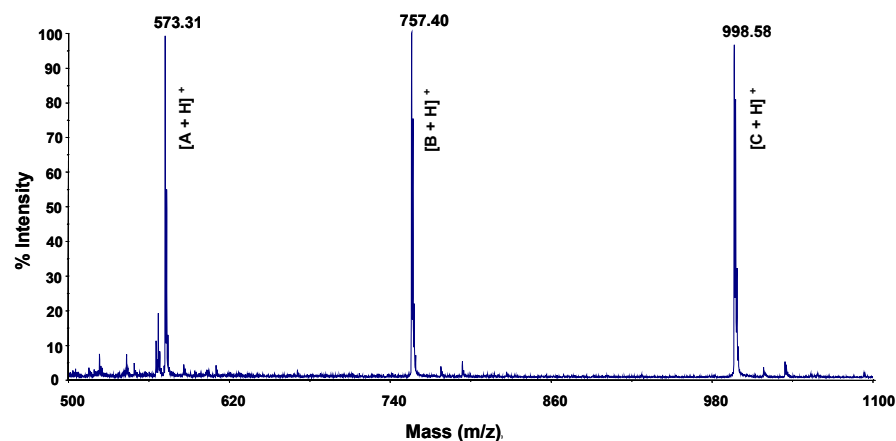
**Note:** The Invitromass™ LMW Calibrant spectra may vary depending on the MALDI instrument and mode of analysis.

The table below lists the final concentration of each peptide/protein in the calibrant after mixing with 24 µl matrix.

Invitromass™ LMW Calibrant	Final Concentration
<b>Calibrant 1</b>	
Bradykinin fragment (1-5)	0.606 pmole/µl
Bradykinin fragment (1-7)	0.67 pmole/µl
Lys(-Des-Arg <sup>9</sup> , Leu <sup>8</sup> )-bradykinin	0.37 pmole/µl
<b>Calibrant 2</b>	
Lys(-Des-Arg <sup>9</sup> , Leu <sup>8</sup> )-bradykinin	0.89 pmole/µl
ACTH (1-16)	0.44 pmole/µl
ACTH (1-24)	0.67 pmole/µl
<b>Calibrant 3</b>	
ACTH (1-24)	0.27 pmole/µl
ACTH (1-39)	0.61 pmole/µl
Insulin	1.12 pmole/µl
<b>Calibrant 4</b>	
Insulin	0.17 pmole/µl
Ubiquitin	1.00 pmole/µl
Cytochrome C	0.83 pmole/µl

### Invitromass™ LMW Calibrant 1

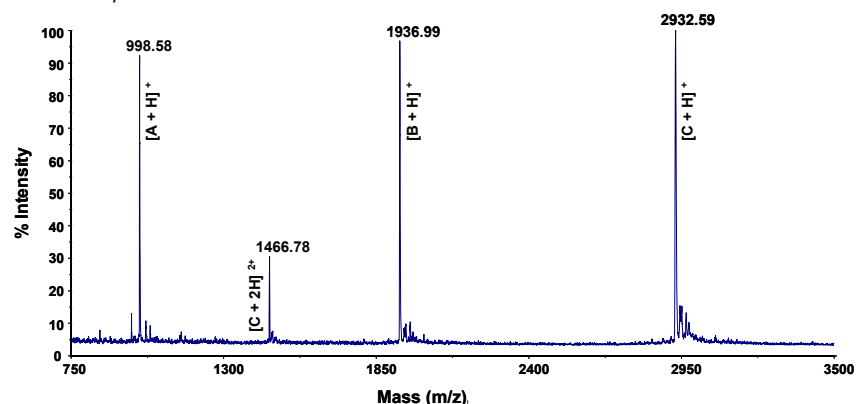
*Monoisotopic mass labels (use monoisotopic mass only when the resolution of the instrument allows you to observe monoisotopic peaks, if not, then use average mass)*



## Expected MALDI-TOF Spectra for Invitromass™ Calibrants, Continued

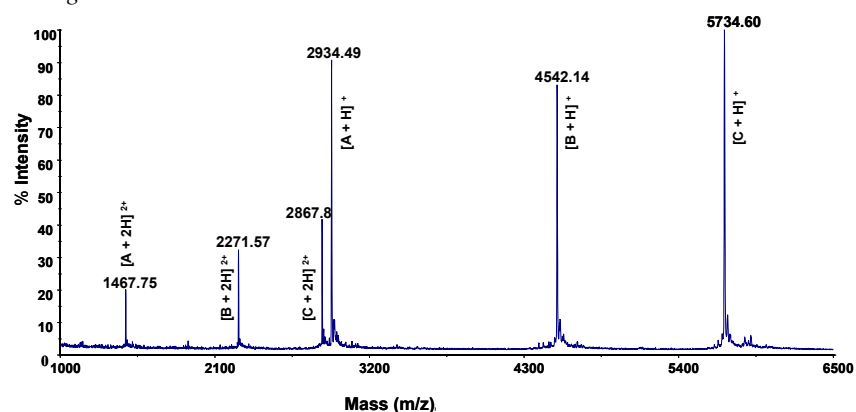
### Invitromass™ Calibrant 2

*Monoisotopic mass labels*



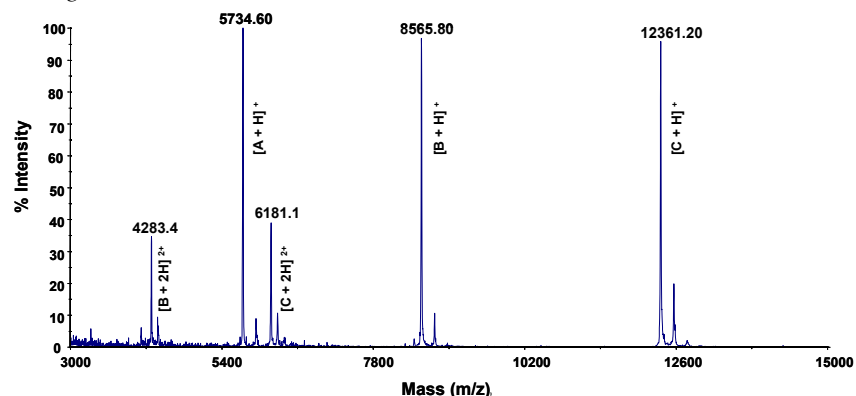
### Invitromass™ Calibrant 3

*Average mass labels*



### Invitromass™ Calibrant 4

*Average mass labels*



## Product Qualification

The Invitromass™ LMW 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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