

## Product Information

### ProteoMass™ Protein MALDI-MS Calibration Kit

Catalog Number **MSCAL3**  
Store at Room Temperature

## TECHNICAL BULLETIN

### Product Description

This kit provides a range of standard proteins for the purpose of calibrating and testing matrix assisted laser desorption ionization (MALDI) mass spectrometers, regardless of the instrument manufacturer. High purity, low alkali metal solvents and a recrystallized matrix are supplied. Whether you are a user new to the interpretation of mass spectrometric data or an experienced biochemist running high throughput experiments for proteomics analysis, this kit is ideally designed to provide standards for most protein applications.

#### Examples of applications:

- Calibration of the MALDI instrument:
  - A mixture of proteins allows for calibration over a wide mass range in linear mode (5,000 up to 67,000 Da) depending on the combination of proteins.
- Tuning of the MALDI Instrument:
  - Combinations of proteins allow for optimization of resolution in linear mode.
- Sensitivity:
  - Sensitivity of the instrument may be tested by using a dilution series of a protein provided by the kit in the mass range of interest.

### Storage/Stability

The kit is stored at room temperature and is shipped at ambient temperature. The matrix, after reconstitution in solvent, is stable for approximately one week at room temperature, if protected from light. Protein stock solutions can be frozen in aliquots, but should not be subjected to more than 3 freeze-thaw cycles. Standards are recommended for use for no longer than one month after reconstitution.

### Components

The standards, matrix, and solvents supplied in the kit may also be purchased as individual components using the catalog numbers listed in the following tables. ProteoMass Peptide & Protein (Catalog No. MSCAL1) and ProteoMass Peptide (Catalog No. MSCAL2) MALDI-MS Calibration Kits are also available.

#### Standard Proteins

2 × 10 nmoles of the standard proteins are supplied in 1.5 ml clear tubes.

Catalog No. [EC or CAS number]	Product	(M+H) <sup>+</sup> Average
I6279 [11070-73-8]	Insulin (bovine)	5,735
C8857 [9007-43-6]	Cytochrome c (equine)	12,362
A8971 [9008-45-1]	Apomyoglobin (equine)	16,952
A9096 [4.1.2.13]	Aldolase (rabbit muscle)	39,212
A8471 [9048-46-8]	Albumin (bovine serum)	66,430

Masses were calculated based on NCBI<sup>1</sup> sequences using NIST standard atomic weights and isotopic masses.<sup>2</sup>

#### Matrix

8 × 10 mg of the recrystallized matrix are supplied in 2.0 ml amber tubes.

Catalog No. [CAS number]	Product	Common Name
S8313 [530-59-6]	3,5-Dimethoxy- 4-hydroxycinnamic acid	Sinapinic acid

### Solvents

Solvents are supplied in high density polyethylene bottles.

Catalog No. [CAS number]	Product	Amount
T3443 [76-05-1]	0.1% Trifluoroacetic acid (TFA) solution	30 ml
A8596 [75-05-8]	Acetonitrile (ACN)	30 ml
T3693 [76-05-1]	1% Trifluoroacetic acid (TFA) solution	4 ml

### **Precautions and Disclaimers**

All the standards have been tested on the Shimadzu Biotech Kompact SEQ and AXIMA-CFR to meet certain performance criteria in the linear positive ion mode of MALDI mass spectrometric analysis. This does not preclude the use of these standards in other modes (i.e., negative ion mode) or with instruments made by other manufacturers. These criteria are only a guideline and not a guarantee of performance on other instrument manufacturers' systems. Performance varies depending on the age and maintenance of the instrument in addition to the manufacturer's own specifications.

### Handling Dilute Protein Solutions

Care is required in the preparation of a dilution series of proteins due to their nature to bind to surfaces.

Therefore, it is recommended to use a new pipette tip for each dilution to avoid carryover. In addition, the most dilute solutions (100 and 10 fmol/ $\mu$ l) will remain useful for only one day, as the sample becomes adsorbed onto the tube surface. The nature of MALDI mass spectrometry excludes the precoating of tubes and tips with bovine serum albumin or fetal calf serum. It is possible to include in the solvents one of a highly limited group of detergents, such as 0.1% octyl- $\beta$ -D-glucopyranoside (Catalog No. O9882),<sup>3,4</sup> to stabilize the solutions, but some affect on the performance of the standards in MALDI mass spectrometry may be observed.

### **Preparation Instructions**

#### Preparation of solvents

- The 0.1% TFA solution is provided ready for use in the preparation of all the standard solutions, except bovine insulin.
- The 1% TFA solution is provided ready for use in the preparation of bovine insulin solutions.
- Mix 5 ml of the 0.1% TFA and 5 ml of ACN to give a solution of 50% ACN in 0.05% TFA. This solvent is used in the preparation of the matrix.

### Preparation of standard stock solutions

**Note:** The user is provided with two options in the preparation of standard solutions depending on preference. Each standard can be prepared as a stock solution of 100 or 10 pmol/ $\mu$ l. Sufficient volume of the 0.1% TFA solution is provided for five preparations of 10-fold serial dilution to 10 fmol/ $\mu$ l in each option.

- For a stock solution of 100 pmol/ $\mu$ l, dissolve the contents of each standard tube in 100  $\mu$ l of the appropriate solvent. (Insulin is dissolved in the 1% TFA solution. The remaining standards are dissolved in the 0.1% TFA solution.)
- For a stock solution of 10 pmol/ $\mu$ l, dissolve the contents of each standard tube in 1000  $\mu$ l of the appropriate solvent.
- Store frozen – recommended to be used for 1 month with a maximum of 3 freeze-thaw cycles before discarding.

### Preparation of solutions for sensitivity analysis

Serially dilute any of the 100 pmol/ $\mu$ l or 10 pmol/ $\mu$ l stock solutions with the appropriate solvent to produce the following working solutions for sensitivity testing.

Initial Concentration	$\mu$ l stock solution	$\mu$ l solvent	Working Solutions
100 pmol/ $\mu$ l	10 $\mu$ l	90 $\mu$ l	10 pmol/ $\mu$ l
10 pmol/ $\mu$ l	10 $\mu$ l	90 $\mu$ l	1 pmol/ $\mu$ l
1 pmol/ $\mu$ l	10 $\mu$ l	90 $\mu$ l	100 fmol/ $\mu$ l
100 fmol/ $\mu$ l	10 $\mu$ l	90 $\mu$ l	10 fmol/ $\mu$ l

### Preparation of solutions for calibration

Beginning with the 100 pmol/ $\mu$ l or 10 pmol/ $\mu$ l stock solutions, prepare a calibration mixture by combining appropriate proteins for the mass range of interest and dilute to a suitable concentration. Typical calibration solution concentrations range between 1–10  $\mu$ M (pmol/ $\mu$ l) for each component. Higher concentrations of larger molecular mass species in the protein mixtures may be necessary to optimize signal intensities across the mass range of interest. For the best mass accuracy, bracket the mass range of interest and, when possible, use three to four proteins for calibration. Serially dilute as described above if desired.

### Preparation of the MALDI Matrix

Dissolve the contents of a 10 mg tube of matrix in 1 ml of the 50% ACN in 0.05% TFA solution. For best performance, once in solution, the matrix should be stored in the dark and used for 1 week, then discarded. Using the 50% ACN in 0.05% TFA solvent, the sinapinic acid forms a nearly saturated solution at room temperature. Some residual crystals may be visible in the matrix solution. The ACN concentration can be adjusted to suit individual preferences. A mixture of 70% ACN and 30% of the 0.1% TFA solution is often used.

### **Procedure**

#### MALDI sample preparation and application to the target

The following methods are provided for the preparation of standards or samples with the matrix for application to the MALDI target. These are general guidelines and not all solvents recommended for the different techniques are supplied with the kit. Typical molar ratios of sample to matrix are between 1:100 and 1:10,000.

#### Sample Preparation Method 1:

Commonly referred to as the dried-droplet method, this method is based on the original MALDI experiments and remains the most commonly used method in the mass spectrometry community.<sup>5</sup>

1. Transfer 10  $\mu$ l of the matrix solution to a small tube.
2. Add 1–10  $\mu$ l of the standard/sample to the tube containing the matrix and vortex.
3. Apply 0.5–2  $\mu$ l of the resulting mixture onto the MALDI target and allow to dry.
4. Once the liquid has evaporated, the target is ready for analysis.

#### Sample Preparation Method 2:

Referred to as the overlayer (or two-layer) method, this method is believed to produce a more homogenous sample spot and to improve resolution and mass accuracy, especially for peptides and proteins.<sup>6-9</sup>

#### First layer solution (matrix only)

1. A concentrated (10–50 mg/ml) solution of the matrix is prepared in methanol or acetone for fast evaporation (solvent not supplied).

#### Second layer solution

2. Prepare a 3–10 mg/ml solution of the matrix in a solvent system with an approximate 2:1 ratio of water (or 0.1% TFA solution) to organic solvent (methanol or ACN).
3. Transfer 10  $\mu$ l of the matrix solution from step 2 to a small tube.

4. Add 1–10  $\mu$ l of the standard/sample to the tube containing the matrix and vortex.

#### Sample deposition

5. Apply 0.5–2  $\mu$ l of the first layer solution (matrix only) to the MALDI target and allow it to dry and form a fine crystalline layer.
6. Apply 0.5–2  $\mu$ l of the second layer solution on top of the crystalline layer and allow to dry. The solvent system used in the second layer solution must not fully dissolve the first layer upon application.
7. Once the liquid has evaporated, the target is ready for analysis.

#### Sample Preparation Method 3:

An alternative method of sample preparation, which eliminates the mixing of the sample with the matrix prior to application to the MALDI target (recommended by Shimadzu Biotech).

1. Apply 0.5  $\mu$ l of matrix solution to the sample deposit area or well of the MALDI target. Remove excess matrix after 1 to 2 seconds and discard. Allow the target surface to dry.
2. Apply 0.5  $\mu$ l of the standard/sample solution to the sample deposit area.
3. While the sample deposit is still wet, add a further 0.5  $\mu$ l of matrix and allow to dry passively.
4. Once all the standards and samples have been applied and allowed to dry, the target is ready for analysis.

### **Product Profile**

Product	NCBI <sup>1</sup> Reference (ExpASY Reference) <sup>10</sup>	Formula (M+H) <sup>+</sup>
Insulin	INS_BOVIN (P01317)	C <sub>254</sub> H <sub>378</sub> N <sub>65</sub> O <sub>75</sub> S <sub>6</sub>
Cytochrome c	CYC_HORSE (P00004)	C <sub>560</sub> H <sub>876</sub> N <sub>148</sub> O <sub>156</sub> S <sub>4</sub> Fe
Apomyoglobin	MYG_HORSE (P68082)	C <sub>769</sub> H <sub>1213</sub> N <sub>210</sub> O <sub>218</sub> S <sub>2</sub>
Aldolase	ALDOA_RABIT (P00883)	C <sub>1733</sub> H <sub>2774</sub> N <sub>489</sub> O <sub>525</sub> S <sub>11</sub>
Albumin	ALBU_BOVIN (P02769)	C <sub>2935</sub> H <sub>4583</sub> N <sub>780</sub> O <sub>899</sub> S <sub>39</sub>

## Results

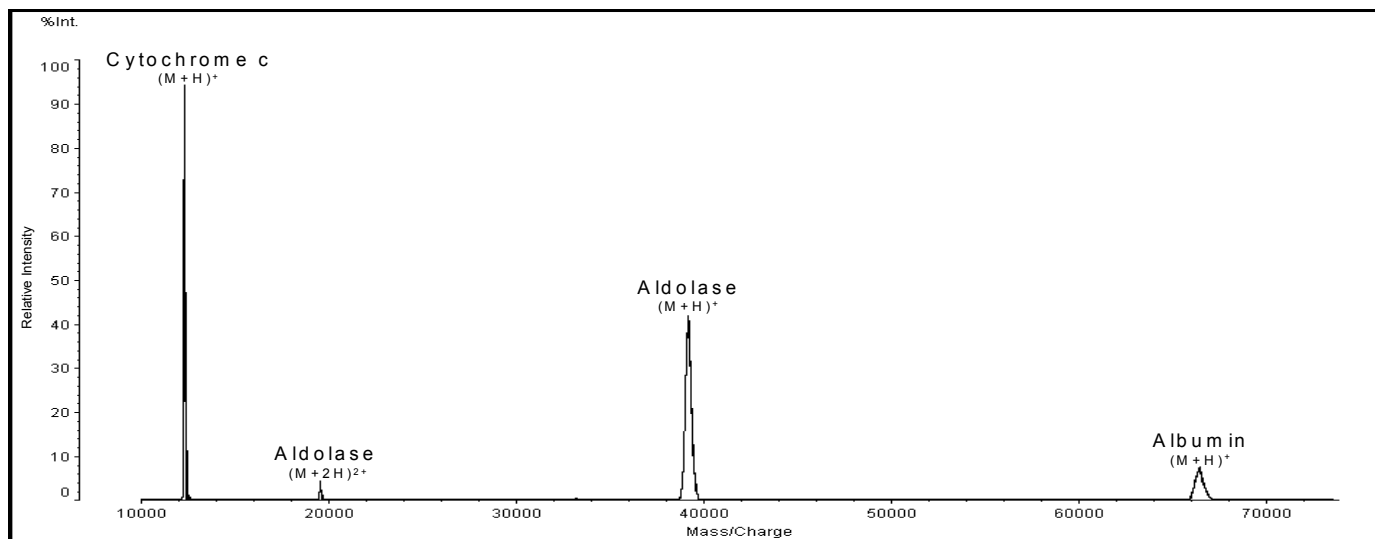


Figure 1. MALDI mass spectrum of a protein calibration solution containing 1.0  $\mu\text{M}$  cytochrome c, 2.0  $\mu\text{M}$  aldolase, and 10  $\mu\text{M}$  albumin. A 10  $\mu\text{l}$  aliquot of the protein solution in 0.1% TFA was mixed with 10  $\mu\text{l}$  of a 10 mg/ml sinapinic acid solution in 70% ACN in 0.03% TFA. 0.8  $\mu\text{l}$  of the resulting solution was spotted onto the MALDI target. Data was acquired using a Shimadzu Biotech Kompact SEQ system in the linear positive ion mode. **Note:** 1  $\mu\text{M}$  = 1 pmol/ $\mu\text{l}$ .

## References

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