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Product Information

ProteoMass™ Peptide & Protein MALDI-MS Calibration Kit

Catalog Number **MSCAL1**
Store at Room Temperature

TECHNICAL BULLETIN

Product Description

This kit provides a range of standard peptides and 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 recrystallized matrices 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 peptide/protein applications.

Examples of applications:

1. Calibration of the MALDI instrument:
   - A combination of peptides provides good calibration across the typical mass range of tryptic digestion fragments (800–3,000 Da) in reflectron mode.
   - 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.
   - Angiotensin II and $P_{14}R$ provide multiple post source decay (PSD) fragment ions for calibration of PSD data.
2. Tuning of the MALDI Instrument:
   - Combinations of peptides or proteins allow for optimization of resolution in reflectron and linear modes, respectively.
3. Sensitivity:
   - Sensitivity of the instrument may be tested by using a dilution series of a peptide or protein provided by the kit in the mass range of interest.

Components

All of the standards supplied in the kit may also be purchased individually using the listed catalog numbers.

Standard Peptides and Proteins

Standard peptides and proteins are supplied in 1.5 ml clear tubes, each containing 10 nmoles of standard.

Catalog No. [EC or CAS number]	Product	$(M+H)^+$ Monoisotopic or Average
B4181 [23815-87-4]	Bradykinin fragment 1-7	757.3997 (Mono)
A8846 [68521-88-0]	Angiotensin II (human)	1,046.5423 (Mono)
P2613	$P_{14}R$ (synthetic peptide)	1,533.8582 (Mono)
A8346 [53917-42-3]	ACTH fragment 18-39 (human)	2,465.1989 (Mono)
I6154 [30003-72-6]	Insulin oxidized B chain (bovine)	3,494.6513 (Mono)
I6279 [11070-73-8]	Insulin (bovine)	5,730.6087 (Mono) 5,735 (Ave)
C8857 [9007-43-6]	Cytochrome c (equine)	12,362 (Ave)
A8971 [9008-45-1]	Apomyoglobin (equine)	16,952 (Ave)
A9096 [4.1.2.13]	Aldolase (rabbit muscle)	39,212 (Ave)
A8471 [9048-46-8]	Albumin (bovine serum)	66,430 (Ave)

Masses were calculated based on NCBI[1] sequences using NIST standard atomic weights and isotopic masses.[2]

Matrices
4 × 10 mg of each recrystallized matrix are supplied in 2.0 ml amber tubes.

Catalog No. [CAS number]	Product	Common Name
C8982 [28166-41-8]	α-Cyano-4-hydroxycinnamic acid	α-cyano CHCA
S8313 [530-59-6]	3,5-Dimethoxy-4-hydroxycinnamic acid	Sinapinic acid

Solvents
The TFA solvents are supplied in high density polyethylene bottles. The acetonitrile is supplied in a glass bottle.

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

ProteoMass Peptide (Catalog No. MSCAL2) and ProteoMass Protein (Catalog No. MSCAL3) MALDI-MS Calibration Kits are also available.

Storage/Stability
The kit is stored at room temperature and is shipped at ambient temperature. Matrices, after reconstitution in solvent, are stable for approximately one week at room temperature, if protected from light. Peptide or 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.

Precautions and Disclaimer
All the standards have been tested on the Shimadzu Biotech Kompact SEQ and AXIMA-CFR to meet certain performance criteria in selected modes of positive ion MALDI mass spectrometric analysis (linear, reflectron, or PSD). 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 Peptide and Protein Solutions
Care is required in the preparation of a dilution series of peptides and 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/µl) 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-β-D-glucopyranoside (Catalog No. O9882),[3,4] 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 bradykinin, insulin oxidized B chain, and bovine insulin.
- 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 bradykinin and insulin oxidized B chain solutions and the matrices.
- The 1% TFA solution is provided ready for use in the preparation of bovine insulin solutions.

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/µl. Sufficient volume of the 0.1% TFA solution is provided for five preparations of 10-fold serial dilution to 10 fmol/µl in each option.
- For a stock solution of 100 pmol/µl, dissolve the contents of each standard tube in 100 µl of the appropriate solvent. (Bradykinin and insulin oxidized B chain are dissolved in the 50% ACN with 0.05% TFA, and insulin is dissolved in the 1% TFA solutions. The remaining standards are dissolved in the 0.1% TFA solution.)
- For a stock solution of 10 pmol/µl, dissolve the contents of each standard tube in 1,000 µl 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/µl or 10 pmol/µl stock solutions with the appropriate solvent to produce the following working solutions for sensitivity testing.

Initial Concentration	µl stock solution	µl solvent	Working Solutions
100 pmol/µl	10 µl	90 µl	10 pmol/µl
10 pmol/µl	10 µl	90 µl	1 pmol/µl
1 pmol/µl	10 µl	90 µl	100 fmol/µl
100 fmol/µl	10 µl	90 µl	10 fmol/µl

Preparation of solutions for calibration

Beginning with the 100 pmol/µl or 10 pmol/µl stock solutions, prepare a calibration mixture by combining appropriate peptides/proteins for the mass range of interest and dilute to a suitable concentration. Typical calibration solution concentrations range between 1–10 µM (pmol/µl) for each component. Higher concentrations of larger molecular mass species in the peptide/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 peptides/proteins for calibration. Serially dilute as described above if desired.

Preparation of MALDI Matrices

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 matrices should be stored in the dark and used for 1 week, then discarded. Using the 50% ACN in 0.05% TFA solvent, both α-cyano-4-hydroxycinnamic acid and sinapinic acid form nearly saturated solutions 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

Recommended matrix for MALDI sample preparations

Standard Peptide or Protein	Suggested Matrix
Bradykinin fragment 1-7	α-cyano
Angiotensin II	α-cyano
$P_{14}R$	α-cyano
ACTH fragment 18-39	α-cyano
Insulin oxidized B chain	α-cyano
Insulin	α-cyano or sinapinic acid
Cytochrome c	α-cyano or sinapinic acid
Apomyoglobin	α-cyano or sinapinic acid
Aldolase	sinapinic acid
Albumin	sinapinic acid

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.[5]

1. Transfer 10 µl of the appropriate matrix solution to a small tube.
2. Add 1–10 µl of the standard/sample to the tube containing the matrix and vortex.
3. Apply 0.5–2 µl 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.[6-9]

First layer solution (matrix only)

1. A concentrated (10–50 mg/ml) solution of the appropriate 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 appropriate 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 µl of the matrix solution from step 2 to a small tube.
4. Add 1–10 µl of the standard/sample to the tube containing the matrix and vortex.

Sample deposition

5. Apply 0.5–2 µl 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 µl 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:
This alternative method of sample preparation eliminates the mixing of the sample with the matrix prior to application to the MALDI target (recommended by Shimadzu Biotech).

1. Apply 0.5 µl of appropriate 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 µl of the standard solution to the sample deposit area.
3. While the sample deposit is still wet, add a further 0.5 µl 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.

Results

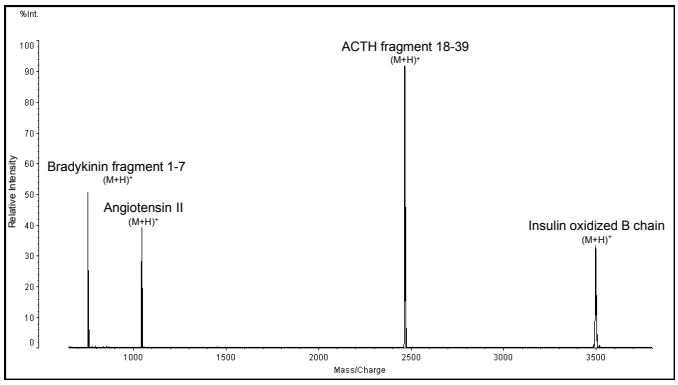

Figure 1. MALDI mass spectrum of a peptide calibration solution containing 1.5 µM bradykinin fragment 1-7, 1.0 µM angiotensin II, 0.5 µM ACTH fragment 18-39, and 2.0 µM insulin oxidized B chain. A 10 µl aliquot of the peptide solution was mixed with 10 µl of a 10 mg/ml α-cyano solution. 0.8 µl of the resulting solution was spotted onto the MALDI target. Data were acquired using a Shimadzu Biotech Kompact SEQ system in the linear positive ion mode. Note: 1 µM = 1 pmol/µl.

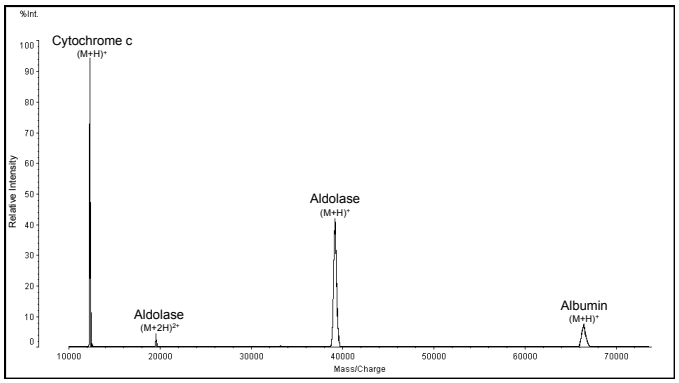

Figure 2. MALDI mass spectrum of a protein calibration solution containing 1.0 μM cytochrome c, 2.0 μM aldolase, and 10 μM albumin. A 10 μl aliquot of the protein solution was mixed with 10 μl of a 10 mg/ml sinapinic acid solution. 0.8 μl of the resulting solution was spotted onto the MALDI target. Data were acquired using a Shimadzu Biotech Kompact SEQ system in the linear positive ion mode. Note: 1 μM = 1 pmol/μl.

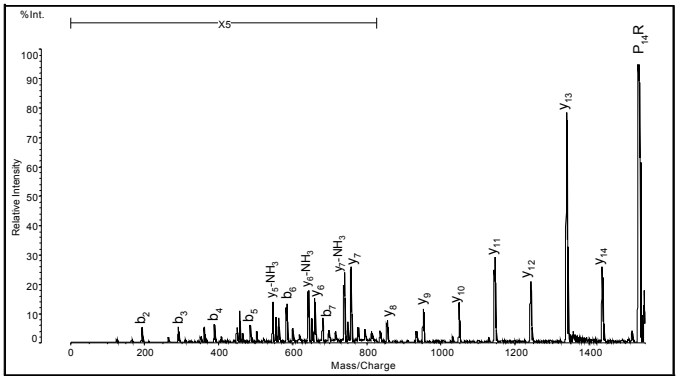

Figure 3. Post Source Decay analysis of $P_{14}R$ using α-cyano as the MALDI matrix. Data were acquired on a Shimadzu Biotech AXIMA-CFR system in reflectron positive ion mode. PSD data compliments of Shimadzu Biotech.

Product Profile

Product	NCBI[1] Reference (ExPASy Reference)[10]	Formula $(M+H)^+$
Bradykinin fragment 1-7	N/A	$C_{35}H_{53}N_{10}O_9$
Angiotensin II	ANGT_HUMAN (P01019)	$C_{50}H_{72}N_{13}O_{12}$
$P_{14}R$	N/A	$C_{76}H_{113}N_{18}O_{16}$
ACTH fragment 18-39	COLI_HUMAN (P01189)	$C_{112}H_{166}N_{27}O_{36}$
Insulin oxidized B chain	INS_BOVIN (P01317)	$C_{157}H_{233}N_{40}O_{47}S_2$
Insulin	INS_BOVIN (P01317)	$C_{254}H_{378}N_{65}O_{75}S_6$
Cytochrome c	CYC_HORSE (P00004)	$C_{560}H_{876}N_{148}O_{156}S_4Fe$
Apomyoglobin	MYG_HORSE (P68082)	$C_{769}H_{1213}N_{210}O_{218}S_2$
Aldolase	ALDOA_RABIT (P00883)	$C_{1733}H_{2774}N_{489}O_{525}S_{11}$
Albumin	ALBU_BOVIN (P02769)	$C_{2935}H_{4583}N_{780}O_{899}S_{39}$

References

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