Max Ion™ Protein MALDI Matrix Kit

Cat. no. MS10005 **Store upon receipt at –20°C**

Contents and Storage

Contents	Quantity	Composition
Sinapinic Acid, Dry Matrix (Starter Sample)	20 mg	Sinapinic acid
Protein Matrix Diluent	5 ml	Proprietary
Invitromass™ Low Molecular Weight (LMW) Calibrant 4 (6,000-12,000 Da)	40 µl	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

The Max Ion™ Protein MALDI Matrix Kit contains sufficient sinapinic acid to analyze ~1,000 spots, and sufficient Protein Matrix Diluent for ~5,000 spots. Additional sinapinic acid can be purchased from Sigma (cat. no. 78867 or 85429). The kit components are guaranteed to be stable for 6 months when stored at –20°C. The sinapinic acid matrix is stable for 2 week at 4°C, once resuspended in the Protein Matrix Diluent.

Description

The Max Ion™ Protein MALDI Matrix Kit is designed for analysis of intact proteins by Matrix-Assisted Laser Desorption Time-Of-Flight mass spectrometry (MALDI-TOF-MS). Sinapinic acid is the matrix of choice for MALDI-TOF analysis of intact large proteins. However, the sinapinic acid has a fragile crystal structure that becomes ablated during prolonged exposure to the MALDI laser. This fragility compromises the signal-to-noise when extended acquisitions are necessary to analyze low-abundance proteins and high molecular weight proteins that ionize inefficiently. The novel formulation of the sinapinic acid in the Max Ion™ Protein MALDI Matrix Kit reduces the laser-induced damage to the crystals, and the proprietary Protein Matrix Diluent enhances the stability and performance of the matrix. Spectra obtained with Max Ion™ Sinapinic Acid Matrix have improved signal-to-noise. Furthermore, the stabilizing effect of Max Ion™ Sinapinic Acid Matrix formulation allows matrix/analyte mixtures spotted on a target to be stored for future analysis—an unprecedented benefit of a MALDI matrix for intact proteins.

The Invitromass™ LMW Calibrant 4 allows accurate mass calibration of peptides, proteins, and organic molecules by MALDI-TOF MS. The Invitromass™ Calibrant is 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.

General Recommendations

- If the analyte sample contains contaminants such as salts, nonvolatile solvents, surfactants, or chaotropes, remove the contaminants by solid-phase extraction or dialysis prior to MALDI-MS analysis.
- Always use polypropylene tubes.
- For MALDI sample spotting, avoid using plastic pipet tips with concentrated acetonitrile (ACN) and trifluoroacetic acid (TFA). We recommend diluting the solvents to working concentrations in glass receptacles washed with 10% acetic acid (HPLC grade) and then dispensing with plastic pipet tips.

Materials Needed

- Sinapinic acid dry matrix (included in the kit as a starter sample)
- Protein Matrix Diluent (included in the kit)
- Protein sample
- MALDI target plate
- Use the Invitromass™ LMW Calibrant 4 included in the kit as an internal standard

Important Note

For best results, prepare the sinapinic acid matrix in the Protein Matrix Diluent supplied with the kit. Do not use any other solvent or diluent to prepare the sinapinic acid matrix.

Reagent Preparation

Sinapinic Acid Matrix

As needed, add 1 ml of Protein Matrix Diluent directly into the tube containing 20 mg Sinapinic Acid Dry Matrix. Vortex for one minute to ensure that the sinapinic acid is completely dissolved. This matrix solution is sufficient for 1,000 spots and is stable for 2 week when stored at 4°C. Additional sinapinic acid can be purchased from Sigma (cat. no. 78867 or 85429). Prepare additional matrix solution by dissolving 20 mg of sinapinic acid in 1 ml of Protein Matrix Diluent.

Invitromass™ LMW Calibrant 4

Prepare a 1:25 dilution of the Invitromass™ LMW Calibrant 4 in the sinapinic acid matrix solution prepared above.

Sample Spotting

Any conventional MALDI spotting method may be used, including the dried droplet, vacuum drying, crushed crystal, fast evaporation, overlayer, sandwich, spin coating, electrospray, quick and dirty, and matrix-precoated plate methods. Spot at least 0.5 µl matrix solution with 0.5 µl (~ 1 pmole) of your protein sample to obtain a good signal. Based on the initial results, you may optimize the amount of protein used for analysis.

Spot 1 µl of calibrant/matrix mixture prepared as above on a MALDI plate to provide a final concentration of 2 pmole of each protein calibrant.

Expected Results

An example of expected results for MALDI-TOF MS analysis of proteins using the Max Ion™ Protein MALDI Matrix Kit is shown.

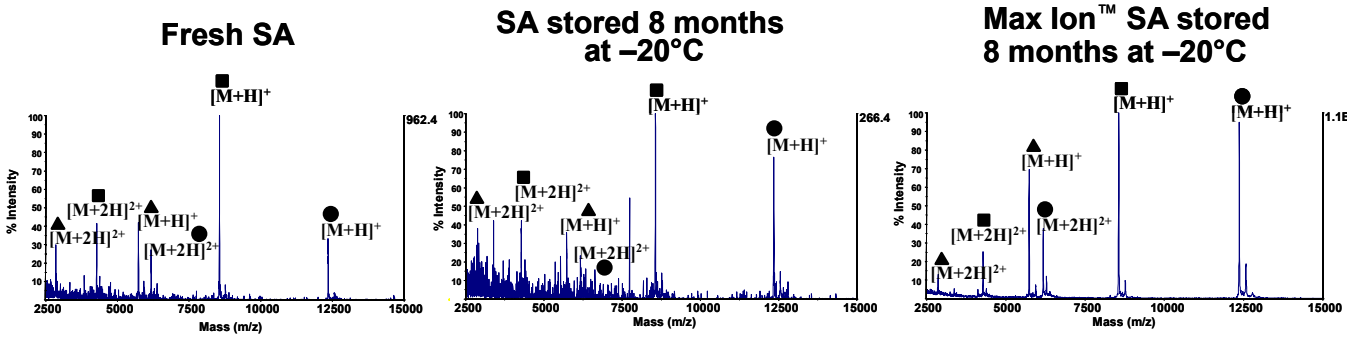

Figure 1. MALDI-MS spectra obtained with 20,000 laser shots of Invitromass™ LMW Calibrant 4 proteins co-spotted with sinapinic acid (SA) or with Max Ion™ sinapinic acid using Voyager-DE™ STR MALDI TOF instrument. The calibrant proteins are insulin, 5,734.6 Da (▲); ubiquitin, 8,565.8 Da (■); and cytochrome c, 12,361.2 Da (●). The spectrum obtained with Max Ion™ sinapinic acid is of very high quality; the matrix resists the loss of signal-to-noise even after the extended exposure to laser irradiation.

The expected average and monoisotopic molecular masses of the singly-charged molecular ions for the Invitromass™ LMW Calibrant 4 components are shown below.

Component	Average Mass $[M+H]^+$	Monoisotopic Mass $[M+H]^+$	Formula
Insulin	5734.60 Da	5730.6087 Da	$C_{254}H_{378}N_{65}O_{75}S_6$
Ubiquitin	8565.80 Da	8560.6250 Da	$C_{378}H_{630}N_{105}O_{118}S$
Cytochrome c	12,361.20 Da	—	$C_{560}H_{874}N_{148}O_{156}S_4Fe$

InvitroMass™ Calibrant 4

Average mass labels

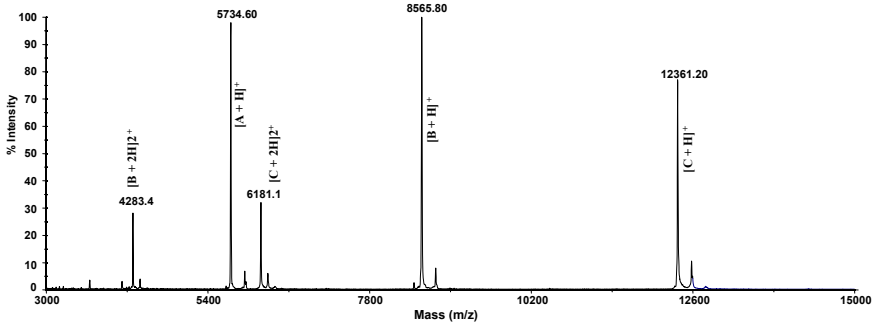

Figure 2. Invitromass™ LMW Calibrant 4 was spotted in Max Ion™ sinapinic acid matrix and analyzed by MALDI-TOF MS in linear mode (5,000 laser shots) using Voyager-DE™ STR MALDI TOF instrument. The spectrum is of high quality, showing defined peaks and accurate molecular masses.

Troubleshooting

Review the information below to troubleshoot your experiments using Max Ion™ Protein MALDI Matrix Kit.

Problem	Solution
No signal, or poor signal intensity: mainly due to improper sample preparation or use of improperly stored materials.	• Prepare the matrix as described in the manual, and spot at least 0.5 µl to obtain a good signal. For best results, always vortex the matrix for 1 minute during preparation. • Store the matrix and the Invitromass™ LMW Calibrant 4 properly, according to instructions. After spotting the Invitromass™ LMW Calibrant 4 on the MALDI plate, perform MALDI-TOF MS analysis the same day. • If you are using the Invitromass™ LMW Calibrant 4 as an internal standard, you may need to optimize the ratio of standard to analyte to obtain comparable results.
High background: originates from the presence of contaminants.	• Always use high-quality, ultrapure reagents for MALDI-TOF MS analysis. • Use clean MALDI plates. • Always use polypropylene tubes.

Product Qualification

The Max Ion™ Protein MALDI Matrix Kit is tested by MALDI-TOF-MS analysis of myoglobin (Sigma) at 1 µM in water with 5 µl of matrix solution as described in this manual by linear positive mode. The $(M+H)^+$ peak at 17,000 Da as well as the $(M+2H)^{2+}$ peak at ~8,600 Da should be discernable.

Products from the Max Ion™ Protein MALDI Matrix Kit are suitable for analysis of intact proteins by MALDI-TOF MS only. This kit has not been qualified for analysis of any other type of analyte or on any other type of MS instrument.

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