

## NEGATIVE ION MODE CALIBRATION USING MASSPREP NEG ION MODE CALIBRANT WITH MALDI-TOF MS

Joomi Ahn, Ying Qing Yu, and Martin Gilar  
Waters Corporation, 34 Maple St. Milford, MA

### INTRODUCTION

MALDI-TOF MS has been widely used as a very practical tool for mass analysis of biopolymers, such as peptides and oligosaccharides. Most analysis is performed in positive ion reflectron mode. Analytes such as acidic peptides, phosphopeptides and sialylated glycans have better ionization efficiency in negative ion mode, therefore, it is ideal to analyze them in the negative ion mode to obtain higher ion counts. Waters has developed a high purity calibration standard under the trade name of MassPREP™ Neg Ion Mode Calibrant for accurate negative ion mass calibrations. This standard provides polymeric peaks across the mass range of 700 to 4,000 amu. Sample preparation is made simple using the standard. This document demonstrates how MassPREP Neg Ion Mode Calibrant should be prepared for MALDI MS analysis. A general guideline on how to perform negative ion data acquisitions using Waters MALDI micro MX™ is also included.

### MATERIALS

- Waters MassPREP Neg Ion Mode Calibrant (2AA-Dextran) 100µg lyophilized solids/vial
- Waters MassPREP MALDI Matrix DHB, 10 mg/vial
- Ammonium tris-citrate (Sigma-Aldrich)
- Acetonitrile (High purity grade, J.T Baker)
- High purity Milli-Q® water
- Waters MALDI Plate
- Glu-fibrino peptide (Glu-Fib, Sigma-Aldrich)

### CALIBRANT PREPARATION

- 1) Make a 1:1 mixture of acetonitrile and H<sub>2</sub>O to reconstitute the lyophilized calibrant.
- 2) Add 50 µL of the solution to the vial containing the lyophilized calibrant.
- 3) Vortex the vial for several seconds to ensure that the solid is completely dissolved.
- 4) Mix equal volumes of sample solution with 2,5-dihydroxybenzoic acid (DHB) matrix solution (40 mg/mL of DHB in solution with high organic content with 8 mM ammonium tris-citrate) in a separate sample container and vortex.
- 5) Apply 1 µL of the mixture onto a clean MALDI target.
- 6) Dry before submitting target for MALDI analysis.

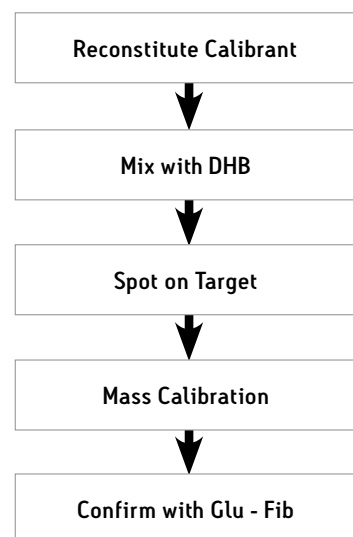


Figure 1. Calibrant Preparation

Note: Optimization for specific applications is recommended. Other relative ratios and solvents may be used for reconstitution.

## EXPERIMENTAL

### Waters MALDI micro MX

- Acquire data in negative mode in MassLynx™ 4.0.
- Run calibrant using settings below in reflectron mode.
- Pulse Voltage = 1950V
- Detector Voltage = 2400V
- Flight Tube = 15,000V
- Negative Anode = 5000V
- Mass Range = 700-5000 amu
- Laser Firing Rate = 10Hz, 10 shots/spectrum
- Adjust laser power at 220 to 240. Aim the laser at different positions for “sweet” spots.
- After acquiring the spectrum, use the peak list (Table 1) for mass calibration.

### Glu-fibrino peptide for mass confirmation

- Mix 20 fmol of glu-fibrino peptide with equal volume of 10 mg/mL DHB.
- Spot 1  $\mu$ L glu-fibrino peptide mix on four neighboring wells of lock mass where the calibrant was calibrated.
- Acquire data in negative ion mode under the same condition after calibration.

A MALDI mass spectrum of the calibrant is shown in the following page (Figure 2). The mass accuracy of the instrument after calibration is shown in Figure 3 using glu-fibrino peptide as a test sample.

## SUMMARY

- Waters MassPREP Neg Ion Mode Calibrant achieves great mass accuracy with Waters MALDI micro MX in 700-4000 amu. RMS error (n=4) was calculated to be 24 ppm for glu-fibrino peptide.
- Performing accurate mass calibration in negative ion mode increases confidence in research results.
- Waters introduces MassPREP MALDI Calibration Kit that includes high purity DHB, CHCA matrices, Reflectron Calibrant Mix, DIOS low mass Calibrant Mix, Neg Ion Mode Calibrant and ACTH.

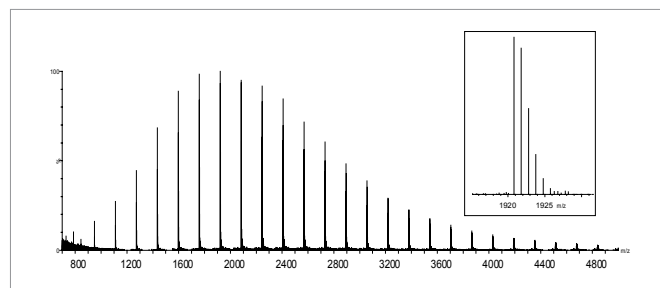


Figure 2. MALDI-TOF MS spectrum of Waters MassPREP Neg Ion Mode Calibrant in negative ion mode between 700-4000 amu.

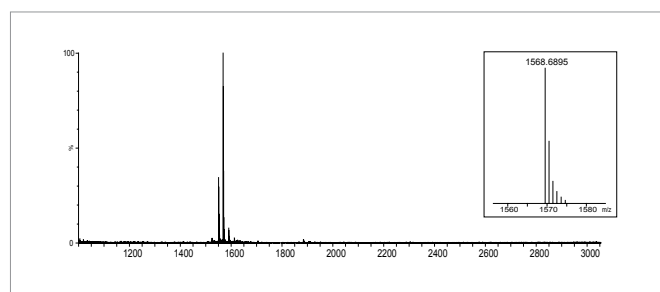


Figure 3. MALDI-TOF MS spectrum of Glu-Fib peptide after applying the calibration; (M-H)<sup>+</sup>theoretical = 1568.677 m/z. Calculated RMS error (n=4) was 24 ppm.

Table 1. Peak List for MassPREP Neg Ion Mode Calibrant

Number of hexose, n=	M-H (m/z)	Number of hexose, n=	M-H (m/z)
4	786.26684	15	2568.84764
5	948.31964	16	2730.90044
6	1110.37244	17	2892.95324
7	1272.42524	18	3055.00604
8	1434.47804	19	3217.05884
9	1596.53084	20	3379.11164
10	1758.58364	21	3541.16444
11	1920.63644	22	3703.21724
12	2082.68924	23	3685.27004
13	2244.74204	24	4027.32284
14	2406.79484	–	–

## ORDERING INFORMATION

Product Description	Part Number
MassPREP Neg Ion Mode Calibrant (1 pk)	186003973
MassPREP MALDI Matrix DHB (5 vials/pk)	186002333

## Other MassPREP Products Ordering Information

Product Description	Part Number
MassPREP Calibrant Mix – MALDI R (1 pk)	186002819
MassPREP Calibrant Mix – DIOS Low Mass (1 pk)	186002820
MassPREP ACTH	186003931
MassPREP MALDI Matrix CHCA (5 vials/pk)	186002331
MassPREP MALDI Calibration Kit	186003927
MassPREP Phosphopeptide Enrichment Kit	186003864

# Waters

THE SCIENCE OF WHAT'S POSSIBLE.™



Waters, The Science of What's Possible, MassPREP, MassLynx and MALDI Micro MX are trademarks of Waters Corporation. Milli-Q is a trademark of Millipore Corporation.

©2007 Waters Corporation. Produced in the U.S.A.  
September 2007 720002340EN SC-PDF

Waters Corporation  
34 Maple Street  
Milford, MA 01757 U.S.A.  
T: 1 508 478 2000  
F: 1 508 872 1990  
[www.waters.com](http://www.waters.com)