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Instructions for Use

Protein Standard II

Protein mixture for testing and calibration of matrix-assisted laser desorption and ionization time-of-flight mass spectrometers (MALDI-TOF MS)

Product description

This protein mixture is designed for testing of MALDI-TOF mass spectrometers in a mass range between ~10,000 and 70,000 Da. The mixture contains three standard proteins. The compounds are listed in the following table together with their calculated mass numbers.

Using the signals of trypsinogen and protein A, the mixture also allows an instrument calibration between ~ 10 and 50 kDa. For instrument calibrations at higher m/z range, extrapolate a linear calibration based on the signals at m/z 22307, 23982 and 44613 (s. Fig. 1b).

Standard proteins are supplied in five tubes per package. The quantity of substance allows 5x250 measurement points.

Note: Bovine serum albumin is not recommended for calibration purposes due to a mass shift to higher m/z values due to protein heterogeneity and adduct formation (s. Fig. 1).

The standard is for research use only. It is not for use in diagnostic procedures.

Proteins		Average m/z
Trypsinogen	$[M+H]^+$	23982
Protein A	$[M+H]^+$	44613
Albumin-Bovine (BSA)	$[M+H]^+$	approx. 66.5 kDa
Protein A	$[M+2H]^{2+}$	22307
Albumin-Bovine (BSA)	$[M+2H]^{2+}$	approx. 33.3 kDa

Storage and stability

The tube box is shipped at ambient temperatures. Store the proteins in the freezer below 0 °C on arrival. Dissolved proteins should be aliquoted and frozen. We do not recommend refreezing dissolved samples after thawing.

Note:	Do not apply repeated freeze-thaw cycles to all materials.

Risk and safety information

The product has to be labeled due to the calculation procedure of the "General Classification guideline for preparations of the EU" in the latest valid version. It contains a harmful component. Please read and observe the Material Safety Data Sheet which is available for download at www.bruker.com/msds in the product description area.

Beside the kit components, we recommend further chemicals within these Instructions for Use. Please read and observe the respective Material Safety Data Sheet to be provided by your supplier. Observe the general safety regulations when handling chemicals.

Sample preparation procedure (recommendation)

Cautionary remarks
Poor sample preparation affects sensitivity, yield low resolution and poor reproducibility. The generation of ions through MALDI depends on a suitable matrix / analyte mixture. For best results use only chemicals of highest available purity.

Chemicals and materials required
- Sinapinic acid (SA) (1 g: # 8201345; 5 g: # 8203073)
- Acetonitrile (ACN)
- 0.1%Trifluoro acetic acid (TFA) in ultra pure water
- TA solvent: mixture of ACN and 0.1% TFA in a volume ratio 1:2

: Sinapinic acid is irritant (H: 315, 319, 335; P: 261, 280, 305+351+338, 321, 405, 501), acetonitrile is highly flammable and harmful (H 225, 302, 312+332, 319; P: 210, 280, 305+351+338).

Equipment and tools required: centrifuge, vortex, sonicator, pipettes and pipette tips

1. Preparation of sample solution
Dissolve contents of each tube in 125 µL 0.1 % TFA and vortex for several seconds.

Alternative for dried droplet preparations only which is not compatible with thin layer or double layer preparations: Dissolve a protein mixture (contents of each tube) in 125 µL TA solvent and vortex for several seconds.

Note:	We highly recommend to aliquot the dissolved protein sample into Eppendorf Safe-Lock microcentrifuge tubes to prevent polymer contaminations.

2. Preparation of matrix solution
Prepare a saturated solution of SA in TA solvent at room temperature and sonicate. Spin down excess matrix (5 min at 4000 g) and use only the clear supernatant as matrix solution.

3. Dried Droplet Preparation on MALDI-TOF steel target
Mix equal volumes of protein sample with matrix solution.
Apply 1 µL onto a standard steel target and allow drying at room temperature.

Note: For AnchorChip™ preparation, refer to the AnchorChip Instructions for Use (# 8215344).

Results of measurement of Protein Standard II

The product is tested on Bruker Daltonics MALDI-TOF mass spectrometers. Fig. 1 shows typical spectra obtained from the standard on an ultraflex III applying different calibration methods.

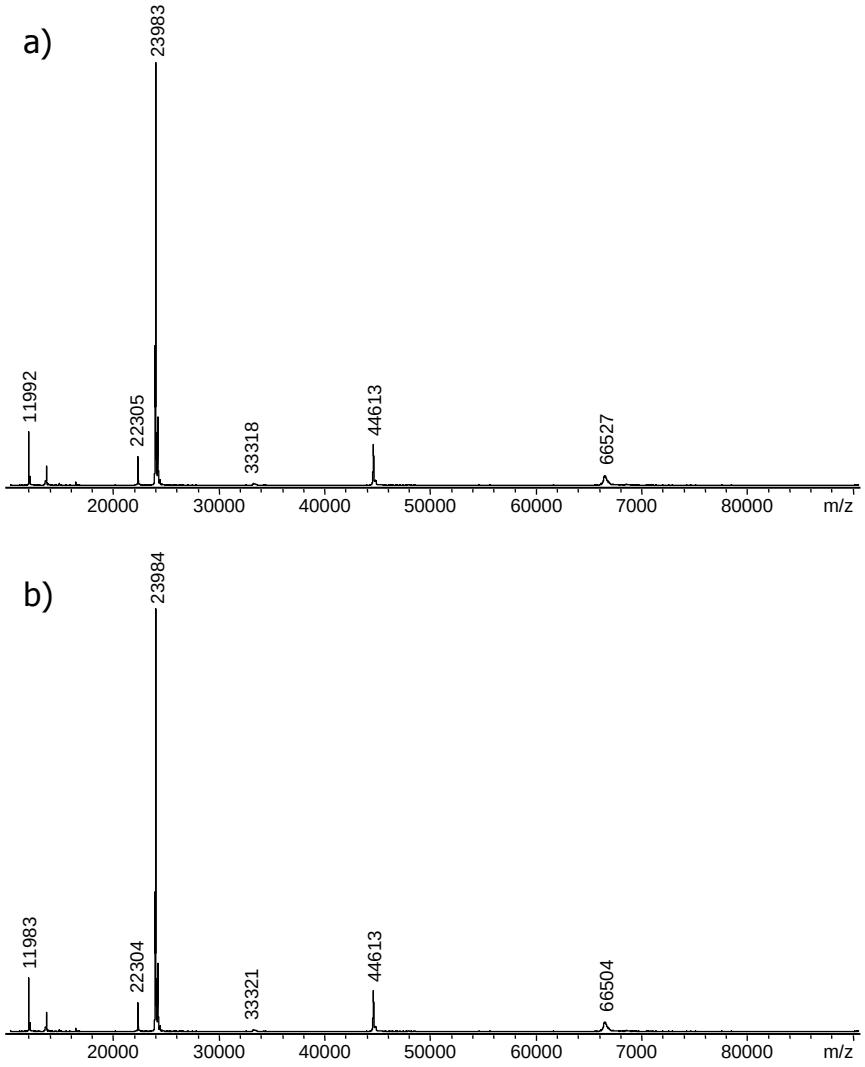

Fig.1: MALDI mass spectrum of Protein Standard II. Target preparation with SA matrix. a: quadratic fit based on protein A and trypsinogen (1- and 2-fold charged), b: linear calibration with protein A (1- and 2-fold charged) and trypsinogen (1-fold charged).

Ordering Information

Product	Part No.
Peptide Calibration Standard	8206195
Peptide Calibration Standard II	8222570
Protein Calibration Standard I	8206355
Protein Standard II	8207234
Sinapinic Acid, 1g	8201345
Sinapinic Acid, 5g	8203073

Bruker Daltonics' Recommendations	
Pipette tips	Eppendorf standard tips
Vials	Eppendorf Safe-Lock microcentrifuge tubes

Support
E-mail: maldi.appl.support@bruker.com
Phone: +49 (421) 2205-184
Fax: +49 (421) 2205-104

Sales Information

Email: care@bruker.com
Phone: +49 (421) 2205-0
Web: www.bruker.com/care

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