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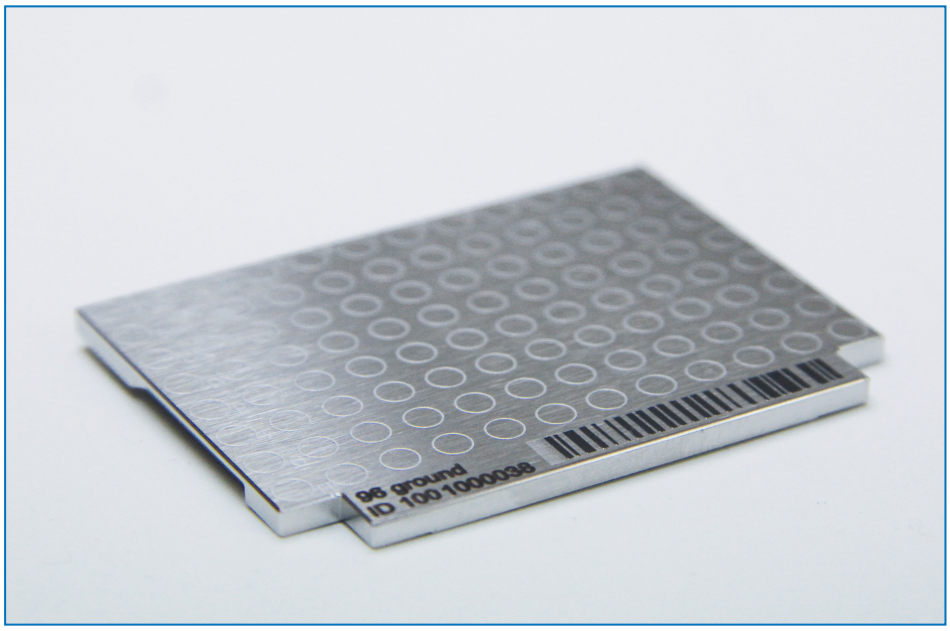
Instructions for Use

Bruker Guide to MALDI Sample Preparation



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Language: en

Revision E (December 2015)

Contents

1 MALDI Target Plate Types

Note This guide is intended as an introduction into MALDI sample preparation in general research applications. Therefore, this guide will focus only on some general MALDI sample preparation protocols using ground steel, AnchorChip and BigAnchor MALDI target plates.

Specialized applications — such as e.g. the identification of microorganisms using the MALDI Biotyper — may require usage of a specific MALDI target plate type and MALDI sample preparation protocol. For more information, see the relevant User Manual or Instructions for Use document.

Ground steel MALDI target plates

Standard MALDI target plate for fast, simple and robust MALDI preparation of virtually any type of sample. These MALDI target plates have a highly regular fine structure on the plate surface, enabling highly homogenous co-crystallized preparations (dried droplet method).

AnchorChip MALDI target plates (anchor diameter 800 µm)

Preferred MALDI target plate type for high-throughput MALDI measurements that are performed in unattended, fully automatic mode. Such applications include MALDI peptide mapping and subsequent MS/MS sequencing of 2D gel digests and LC-MALDI analyses of complex peptide mixtures.

Sample positions on AnchorChip MALDI target plates contain "anchors"; hydrophilic patches surrounded by a hydrophobic ring. The "anchor" localizes droplets at the sample position and the hydrophobic ring prevents sample spreading and concentrates the sample into a spot 800 µm in diameter.

After correct adjustment of the MALDI target plate in the MALDI ion source, the localization effect ensures that every single laser shot fired throughout an automatic run will hit a sample spot. This significantly increases the efficiency of the MALDI acquisition process. The concentration effect also provides enhanced sensitivity when analyzing dilute samples.

BigAnchor MALDI target plates (anchor diameter 2000 µm)

BigAnchor MALDI target plates feature a wider spot diameter (2000 µm). These MALDI target plates are intended for use and provide enhanced preparation quality with MALDI matrices that are difficult to prepare on the narrow spots featured on the 800 µm AnchorChip MALDI target plates (e.g. 2,5-DHAP matrix).

SmallAnchor MALDI target plates (anchor diameter 400 µm)

Preferred MALDI target plate type for the preparation of oligonucleotides and similar samples using 3-HPA as a matrix.

2 Risk and Safety Information

Various chemicals are required for the procedures described in these Instructions for Use.

For information about hazards and precautions related to handling of chemical substances and mixtures always refer to the Material Safety Data Sheets (MSDS) which must be provided by the supplier of the chemicals. Carefully read the Material Safety Data Sheet before handling any substance used in the procedures below. Follow the general safety regulations when handling or disposing of chemicals and biohazardous material. Always use appropriate protective equipment and preferably handle materials in a fume hood.

Material Safety Data Sheets for the Bruker CARE products are available for download at:

http://www.bruker.com/msds

3 How to Clean MALDI Target Plates

Note This protocol can be used to clean MALDI target plates used in general research applications.

Specialized applications — such as e.g. the identification of microorganisms using the MALDI Biotyper — may require that MALDI target plates are cleaned using a dedicated procedure. For more information, see the relevant User Manual or Instructions for Use document.

Chemicals and Materials Required

IMPORTANT Follow the general safety regulations when handling hazardous chemicals or biohazardous material. Also refer to section 2 "Risk and Safety Information".

- 2-propanol
- Deionized water
- Solvent TA30 (30:70 [v/v] Acetonitrile : TFA 0.1% in water)
- Ultrasonic bath
- Clean, high-sided container large enough to accommodate the MALDI target plate
- Lint-free tissues (for example, Kimwipes)

►► Cleaning Procedure

1. Wet a tissue with 2-propanol and wipe the sample/matrix spots from the surface of the MALDI target plate.
2. Wet a tissue with water and wipe the upper surface of the MALDI target plate.
3. Place the MALDI target plate into a clean high-sided container and pour in enough 2-propanol to submerge the MALDI target plate. Place the container in the ultrasonic bath and sonicate for 10 minutes.
4. Place the MALDI target plate into a clean high-sided container and pour in enough solvent TA30 to submerge the MALDI target plate. Place the container in the ultrasonic bath and sonicate for 10 minutes.

5. Dry the MALDI target plate using a stream of high-purity nitrogen or compressed air.

   Do not wipe the upper surface of the cleaned target.

   Note If high-purity gases are not available, allow the plate to dry at ambient temperature in a dust-free environment.

4 MALDI Sample Preparation Protocols

Note The following protocols can be used for MALDI sample preparation in general life science applications.

Specialized applications — such as e.g. the identification of microorganisms using the MALDI Biotyper — may require dedicated MALDI sample preparation procedures. For more information, see the relevant User Manual or Instructions for Use document.

Matrix	Ground steel MALDI target plates	AnchorChip MALDI target plates	BigAnchor MALDI target plates	SmallAnchor MALDI target plates
HCCA	Section 4.1	Sections 4.2 + 4.3	*not applicable*	*not applicable*
2,5-DHB	Section 4.4	Section 4.5	*not applicable*	*not applicable*
2,5-DHAP	Section 4.6	*not applicable*	Section 4.6	*not applicable*
SA	Section 4.7	*not applicable*	*not applicable*	*not applicable*
SDHB	Section 4.8	Section 4.9	Section 4.10	*not applicable*
3-HPA	Section 4.11	Section 4.12	*not applicable*	Section 4.13
1,5-DAN	Section 4.14	*not applicable*	*not applicable*	*not applicable*

IMPORTANT Follow the general safety regulations when handling hazardous chemicals or biohazardous material. Also refer to section 2 "Risk and Safety Information".

HCCA — α-Cyano-4-hydroxycinnamic acid. HCCA enables highly sensitive MALDI-TOF-MS measurement of peptides and proteins from 0.7 to 20 kDa.

2,5-DHB —2,5-Dihydroxybenzoic acid. 2,5-DHB can be used for MALDI-TOF-MS analysis of a wide variety of peptides, proteins, polymers and carbohydrates, including phosphopeptides and glycoproteins.

2,5-DHAP — 2,5-Dihydroxyactetophenone. 2,5-DHAP is a MALDI matrix used for preparations of proteins with a mass of 8–100 kDa. 2,5-DHAP prevents ISD fragmentation and is recommended for proteomic profiling studies and for the analysis of glycoproteins.

SA — Sinapinic acid (*trans*-3,5-dimethoxy-4-hydroxycinnamic acid). SA is a good choice for analysis of larger proteins (10–150 kDa) and some polar polymers. It is also suitable for generation of ISD spectra of intact proteins. Small peptides (<3 kDa) may not produce strong signals with SA, and in such cases we recommend using HCCA as a MALDI matrix.

SDHB — 90:10 mixture of 2,5-DHB and 2-Hydroxy-5-methoxybenzoic acid. We recommend using SDHB MALDI matrix instead of 2,5-DHB for MALDI-TOF-MS analysis of very large proteins and glycoproteins. SDHB is also suitable for the generation of ISD spectra of intact proteins.

3-HPA — 3-Hydroxypicolinic acid. 3-HPA has proved useful as a MALDI matrix material for the analysis of mixed oligonucleotide samples (DNA/RNA) between 1 and 30 kDa.

1,5-DAN — 1,5-Diaminonaphthalene. 1,5-DAN effectively promotes reduction of disulfide bonds in the gas phase. This greatly facilitates analysis of proteins and peptides containing disulfide linkages in top-down sequencing of intact proteins (ISD; T^3).

4.1 HCCA Dried Droplet (Ground Steel MALDI Target Plates)

Sample type

- Peptides, protein digests

Sample solvent

- TA30 solvent (30:70 [v/v] acetonitrile : 0.1% TFA in water) or 0.1% TFA

Matrix solubilization procedure

- Prepare a saturated solution of HCCA in TA30 solvent.

►► Sample preparation

1. Mix 1 part saturated HCCA solution with 1 part sample solution.
2. Deposit 0.5 µL of the matrix/analyte mixture onto the MALDI target plate and allow to dry.

4.2 HCCA Dried Droplet (AnchorChip MALDI Target Plates)

Sample type

- Peptides, protein digests

Sample solvent

- TA30 solvent (30:70 [v/v] acetonitrile : 0.1% TFA in water) or 0.1% TFA

Matrix solubilization procedure

- Prepare a matrix solution of 1.4 mg/mL HCCA dissolved in a solvent mixture containing 85% acetonitrile, 15% water, 0.1% TFA and 1 mM $NH_4H_2PO_4$.

Note Before starting, make sure that the sample does not contain alkaline salts, surfactants or other contaminants that are known to interfere with MALDI. If such contaminants are present, clean up the sample before an acquisition run using ZipTip® pipette tips, dialysis membranes, or similar devices.

►► Sample preparation

1. Deposit 0.5–1 µL of the sample solution onto each MALDI target plate position and allow to dry.
2. Deposit 0.5 µL of the matrix solution onto each sample spot and allow to dry.

►► Preparation of external calibrant spots

1. Dissolve Peptide Calibration Standard II (# 8222570) in 125 µL TA30.
2. Mix 1 part calibrant solution with 200 parts HCCA matrix solution and deposit 0.5 µL of the calibrant/matrix mixture onto calibrant anchor spots on the AnchorChip MALDI target plate.

4.3 HCCA Dried Droplet for nanoLC-MALDI (AnchorChip MALDI Target Plates)

Sample type

- nanoLC-MALDI analysis of peptide mixtures using typical nanoLC flow rates of 300 nL/min

Chemicals and materials required

- TA30 (30:70 [v/v] acetonitrile : 0.1% TFA)
- TA85 (85:15 [v/v] acetonitrile : 0.1% TFA)
- TA90 (90:10 [v/v] acetonitrile : 0.1% TFA)
- TA95 (95:5 [v/v] acetonitrile : 0.1% TFA)
- HCCA stock solution — saturated solution of HCCA in TA90
- 10% TFA
- 100 mM $NH_4H_2PO_4$
- Peptide Calibration Standard II (# 8222570) dissolved in 125 µL TA30

►► Spotting method for nanoLC fractions

1. Prepare 800 µL of matrix solution by mixing:
   - 748 µL TA95
   - 36 µL HCCA stock solution
   - 8 µL 10% TFA
   - 8 µL 100 mM $NH_4H_2PO_4$
2. Set the syringe pump supplying the matrix to a flow rate of 100 µL/h (15 s fractions) or 150 µL/h (10 s fractions).

   In both cases this corresponds to approximately 420 nL matrix solution per spot.

►► Preparation of external calibrant spots

Note The matrix solution used for external calibration spots is prepared using a solvent containing more water than that used for nanoLC fraction matrix solution (TA85 instead of TA95).

1. Prepare 800 µL external calibrant matrix solution by mixing:
   - 748 µL TA85
   - 36 µL HCCA stock solution
   - 8 µL 10% TFA
   - 8 µL 100 mM $NH_4H_2PO_4$
2. Mix 300 µL of the external calibrant matrix solution prepared in step 1 with 1.5 µL Peptide Calibration Standard II solution.
3. Deposit 420 nL calibrant/matrix mixture onto calibrant anchor spots on the AnchorChip MALDI target plate.

4.4 2,5-DHB Dried Droplet (Ground Steel MALDI Target Plates)

Sample type

- Peptides, phosphoprotein digests, glycoprotein digests,intact proteins, glycans

Sample solvent

- TA30 solvent (30:70 [v/v] acetonitrile : 0.1% TFA in water) or 0.1% TFA
  - For glycan analysis, use water as the sample solvent.

Matrix solubilization procedure

- Prepare a matrix solution of 20 mg/mL 2,5-DHB in TA30.
  - For glycan analysis, supplement the matrix solution with 1 mM NaCl.
  - For phosphopeptide analysis, supplement the matrix solution with 1% H_3PO_4.

►► Sample preparation

1. Mix 1 part matrix solution with 1 part sample solution.
2. Deposit 0.5 µL of the matrix/analyte mixture onto the MALDI target plate and allow to dry.

4.5 2,5-DHB Dried Droplet (AnchorChip MALDI Target Plates)

Sample type

- Peptides, glycoprotein digests, glycans

Sample solvent

- TA30 solvent (30:70 [v/v] acetonitrile : 0.1% TFA in water) or 0.1% TFA
  - For glycan analysis, use water as the sample solvent.

Matrix solubilization procedure

- Prepare a matrix solution of 10 mg/mL 2,5-DHB in TA30.
  - For glycan analysis, supplement the matrix solution with 1 mM NaCl.

►► Sample preparation

1. Deposit 0.5 µL of the matrix solution onto each MALDI target plate position and allow to dry.
2. Deposit 0.5–1 µL of the sample solution onto each matrix spot and allow to dry.

4.6 2,5-DHAP Dried Droplet (Ground Steel MALDI Target Plates + BigAnchor MALDI Target Plates)

Sample type

- Intact proteins

Sample solvent

- 0.1% TFA

Matrix solubilization procedure

- Dissolve 7.6 mg 2,5-DHAP in 375 µL ethanol. Add 125 µL of an 18 mg/mL aqueous solution of diammonium hydrogen citrate ($C_6H_8O_7 * 2NH_3$).

►► Sample preparation

1. Mix 2 µL sample solution with 2 µL 2% TFA.
2. Add 2 µL matrix solution and pipette up and down until crystallization starts.
3. Deposit 0.5 µL of the crystal suspension onto the MALDI target plate and allow to dry.

4.7 SA Double Layer (Ground Steel MALDI Target Plates)

Sample type

- Intact proteins

Sample solvent

- 0.1% TFA

Matrix solubilization procedure

- Matrix solution A: prepare a saturated solution of SA in ethanol.
- Matrix solution B: prepare a saturated solution of SA in TA30 solvent (30:70 [v/v] acetonitrile : 0.1% TFA in water).

►► Sample preparation

1. Deposit 0.5 µL matrix solution A onto the MALDI target plate and allow to dry.
2. Mix 1 part matrix solution B with 1 part analyte solution.
3. Deposit 0.5 µL of the matrix/analyte mixture onto the matrix spot and allow to dry.

4.8 SDHB Dried Droplet (Ground Steel MALDI Target Plates)

Sample type

- Intact proteins, top-down sequencing of intact proteins (ISD)

Sample solvent

- TA30 solvent (30:70 [v/v] acetonitrile : 0.1% TFA in water) or 0.1% TFA

Matrix solubilization procedure

- Prepare a matrix solution of 50 mg/mL SDHB in TA50 solvent (50:50 [v/v] acetonitrile : 0.1% TFA in water).

►► Sample preparation

1. Mix 1 part matrix solution with 1 part sample solution.
2. Deposit 0.5 µL of the matrix/analyte mixture onto the MALDI target plate and allow to dry.

4.9 SDHB Dried Droplet (AnchorChip MALDI Target Plates)

Sample type

- Intact proteins, top-down sequencing of intact proteins (ISD)

Sample solvent

- TA30 solvent (30:70 [v/v] acetonitrile : 0.1% TFA in water) or 0.1% TFA

Matrix solubilization procedure

- Prepare a matrix solution of 10 mg/mL SDHB in TA30.

►► Sample preparation

1. Deposit 0.5 µL of the matrix solution onto each MALDI target plate position and allow to dry.
2. Deposit 0.5–1 µL of the sample solution onto each matrix spot and allow to dry.

4.10 SDHB Dried Droplet (BigAnchor MALDI Target Plates)

Sample type

- Intact proteins, top-down sequencing of intact proteins (ISD)

Sample solvent

- TA30 solvent (30:70 [v/v] acetonitrile : 0.1% TFA in water) or 0.1% TFA

Matrix solubilization procedure

- Prepare a matrix solution of 25 mg/mL SDHB in TA50 solvent (50:50 [v/v] acetonitrile : 0.1% TFA in water).

►► Sample preparation

1. Deposit a µL aliquote of the sample solution onto the MALDI target plate position and allow to dry.
2. Deposit 1µl of the matrix solution and allow to dry.

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4.11 3-HPA Dried Droplet (Ground Steel MALDI Target Plates)

Sample type

- Oligonucleotides

Sample solvent

- Water

Matrix solubilization procedure

- Prepare a saturated solution of 3-HPA in TA50 solvent (50:50 [v/v] acetonitrile : 0.1% TFA in water) containing 10 mg/mL diammonium hydrogen citrate ($C_6H_8O_7 * 2NH_3$).

►► Sample preparation

1. Deposit 0.5 µL of the matrix solution onto each MALDI target plate position and allow to dry.
2. Deposit 0.5 µL of the sample solution onto each matrix spot and allow to dry.

4.12 3-HPA Dried Droplet (AnchorChip MALDI Target Plates)

Sample type

- Oligonucleotides

Sample solvent

- Water

Matrix solubilization procedure

- Prepare a half-concentrated solution of 3-HPA in TA50 solvent (50:50[v:v] acetonitrile : 0.1% TFA in water) containing 10mg/mL diammonium hydrogen citrate ($C_6H_8O_7 * 2NH_3$)

►► Sample preparation

1. Deposit 0.5 µL of the matrix solution onto each MALDI target plate position and allow to dry.
2. Deposit 0.5 µL of the sample solution onto each matrix spot and allow to dry.

4.13 3-HPA Dried Droplet (SmallAnchor MALDI Target Plates)

Sample type

- Oligonucleotides

Sample solvent

- Water

Matrix solubilization procedure

- Prepare a matrix solution of 10 mg/mL 3-HPA and 1 mg/mL diammonium hydrogen citrate ($C_6H_8O_7 * 2NH_3$) in water.

►► Sample preparation

1. Deposit 1 µL of the matrix solution onto each MALDI target plate position and allow to dry.
2. Deposit 1 µL of the sample solution onto each matrix spot and allow to dry.

4.14 1,5-DAN Dried Droplet (Ground Steel MALDI Target Plates)

Sample type

- Top-down sequencing of intact proteins (ISD; T^3)

Sample solvent

- TA30 solvent (30:70 [v/v] acetonitrile : 0.1% TFA in water) or 0.1% TFA

Matrix solubilization procedure

- Prepare a saturated solution of 1,5-DAN in TA50 solvent (50:50 [v/v] acetonitrile : 0.1% TFA in water).

Note To avoid degradation of the matrix, always prepare a fresh solution immediately before use.

1,5-DAN is classified as a hazardous chemical; it is suspected of being carcinogenic and is very toxic to aquatic life with long lasting effects.

WARNING (H: 351, 410; P: 273, 281, 501); refer to the MSDS of 1,5-DAN.

►► Sample preparation

1. Mix 2 parts matrix solution with 1 part sample solution.
2. Deposit 0.5 µL of the matrix/analyte mixture onto the MALDI target plate and allow to dry.

5 Ordering Information

Product	Part Number
MALDI target plates	
MSP 96 target ground steel BC	# 8280799
MTP 384 target plate ground steel BC	# 8280784
MSP AnchorChip 96 BC	# 8280823
MTP AnchorChip 384 BC	# 8280790
MTP AnchorChip 1536 BC	# 8280787
MTP BigAnchor 384 BC	# 8280788
MTP SmallAnchor 384 BC	# 8280792
MALDI matrices	
HCCA; α-Cyano-4-hydroxycinnamic acid, 1 g	# 8201344
2,5-DHB; 2,5-Dihydroxybenzoic acid, 1 g	# 8201346
2,5-DHAP; 2,5-Dihydroxyacetophenone, 1 g	# 8231829
SA; Sinapinic acid, 1 g	# 8201345
sDHB, 5 g	# 8209813
3-HPA; 3-Hydroxypicolinic acid, 1 g	# 8201224
MALDI calibration standard	
Peptide Calibration Standard II	# 8222570

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