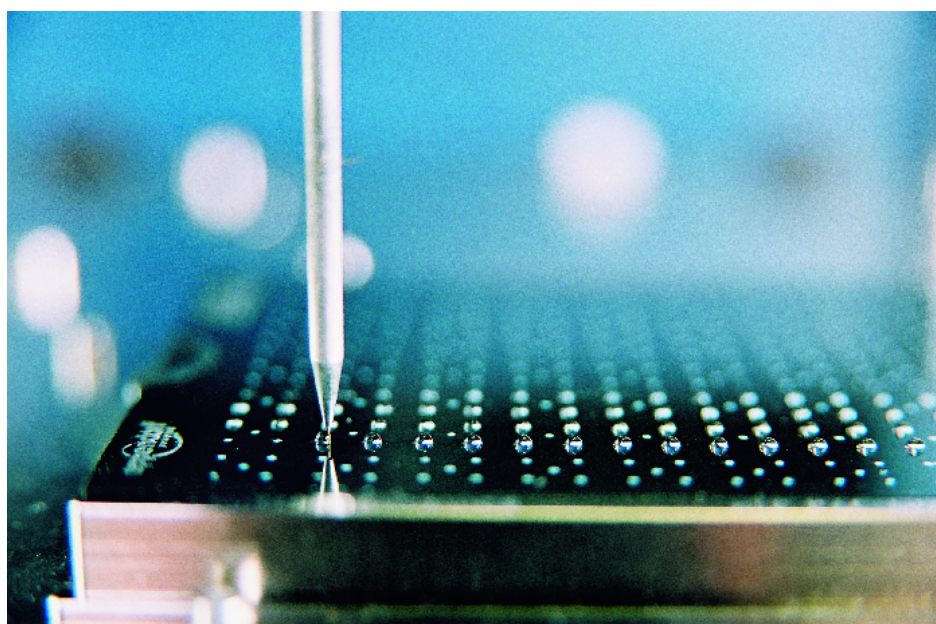


Bruker Guide to MALDI Sample Preparation



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1 MALDI Target Types

Note Specialized applications — such as identification of microorganisms using MALDI Biotyper — may require use of a specific MALDI target type. For more information, see the relevant User Manual or Instructions for Use document.

Steel targets — Standard target for fast, simple and robust MALDI preparation of virtually any type of sample. Two types of steel target are available:

- Ground steel targets have a highly regular fine structure on the plate surface, enabling highly homogenous co-crystallized preparations (dried droplet method).
- Polished steel targets are virtually free of any surface structure, and provide maximum flatness for MALDI thin-layer preparations.

AnchorChip™ Standard targets (Ø = 800 µm) — Preferred target 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 targets 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 target 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.

SmallAnchor targets (Ø = 400 µm) — Preferred target type for the preparation of oligonucleotides and similar samples using 3-HPA as a matrix.

Prespotted AnchorChip (PAC) targets — Disposable MTP-sized MALDI targets prespotted with HCCA (α -cyano-4-hydroxycinnamic acid) matrix. PAC targets are highly suitable for low- to medium-complexity samples in gel- or LC-based proteomics applications.

2 How to Clean MALDI Targets

Note This protocol can be used to clean MALDI targets used in general research applications. Specialized applications — such as identification of microorganisms using MALDI Biotyper — may require that MALDI targets are cleaned using a dedicated procedure. For more information, see the relevant User Manual or Instructions for Use document.

Target type

- Ground Steel / AnchorChip

Chemicals and Materials Required

IMPORTANT Carefully read the Material Safety Data Sheet provided by the supplier and follow general safety regulations when handling chemicals or biohazardous material.

- 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
- Lint-free tissues (for example, Kimwipes)

►► 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 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 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.

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

3 Sample Preparation Protocols

Note These protocols can be used for sample preparation in general research applications. Specialized applications — such as identification of microorganisms using MALDI Biotyper — may require dedicated sample preparation procedures. For more information, see the relevant User Manual or Instructions for Use document.

Matrix	Ground Steel targets	AnchorChip targets	Prespotted AnchorChip (PAC) targets
HCCA	Protocol 3.1	Protocol 3.2 ^a , Protocol 3.3 ^a	See Protocols 3.4 and 3.5
2,5-DHB	Protocol 3.6	Protocol 3.7 ^a	<i>not applicable</i>
2,5-DHAP	Protocol 3.8	<i>not applicable</i>	<i>not applicable</i>
SA	Protocol 3.9	<i>not applicable</i>	<i>not applicable</i>
SDHB	Protocol 3.10	<i>not applicable</i>	<i>not applicable</i>
1,5-DAN	Protocol 3.11	<i>not applicable</i>	<i>not applicable</i>
3-HPA	Protocol 3.12	Protocol 3.13 ^b	<i>not applicable</i>

^a AnchorChip Standard targets ($\varnothing = 800 \mu\text{m}$); ^b SmallAnchor targets ($\varnothing = 400 \mu\text{m}$)

IMPORTANT Carefully read the Material Safety Data Sheet provided by the supplier and follow general safety regulations when handling chemicals or biohazardous material.

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-Dihydroxyacetophenone. 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.

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; T³).

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.

3.1 HCCA Dried Droplet, Ground Steel Targets

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 and allow to dry.

The concentration of the peptide/protein solution should be between 10 fmol – 1 pmol/ μL .

3.2 HCCA Dried Droplet, AnchorChip Standard Targets

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 0.7 mg/mL HCCA dissolved in a solvent mixture containing 85% acetonitrile, 15% water, 0.1% TFA and 1 mM $\text{NH}_4\text{H}_2\text{PO}_4$.

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

▶▶ Preparation of external calibrant spots

1. Dissolve Peptide Calibration Standard II (# 222570) in 125 μL TA30.
2. Mix 1 part calibrant solution with 200 parts HCCA matrix solution and deposit 1 μL of the calibrant/matrix mixture onto calibrant anchor spots on the AnchorChip target.

3.3 HCCA Dried Droplet for nanoLC-MALDI, AnchorChip Standard Targets

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 $\text{NH}_4\text{H}_2\text{PO}_4$
- Peptide Calibration Standard II (# 222570) dissolved in 125 μL TA30

►► Spotting method for nanoLC fractions

1. Prepare 800 μL nanoLC fraction matrix solution by mixing:
 - 748 μL TA95
 - 36 μL HCCA stock solution
 - 8 μL 10% TFA
 - 8 μL 100 mM $\text{NH}_4\text{H}_2\text{PO}_4$
2. Set the syringe pump supplying the matrix to a flow rate of 100 $\mu\text{L}/\text{h}$ (15 s fractions) or 150 $\mu\text{L}/\text{h}$ (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 $\text{NH}_4\text{H}_2\text{PO}_4$
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 target.

3.4 PAC Targets, Affinity Method

This preparation method is based on immobilization of peptides on the pre-coated HCCA matrix spots and provides MS spectra of high signal-to-noise ratios for peptide digests.

Peptides are immobilized by the adsorption equilibrium that is maintained between the peptide sample solution and the outer crystal surface of the HCCA matrix thin layer during the incubation step.

Because this “affinity” method is driven by adsorption equilibria, highly hydrophilic peptides and other peptides that are less efficiently adsorbed on the HCCA matrix crystal surface may be detected in the resulting MALDI spectra at lowered relative intensities, or may not be detected at all.

In general, preparations using this method offer a lower MS/MS capacity per spot compared to the dried sample technique (see section 3.5).

However, this method is more tolerant of contaminants that are typically contained in digest samples (buffer salts, surfactants, and so on). This tolerance may reduce the need for additional sample clean-up steps (for instance, using ZipTips) before spotting.

Sample type

- Peptides, protein digests

Chemicals and materials required

- Washing Buffer — 10 mM ammonium phosphate, monobasic (for example, Sigma-Aldrich A1645) in 0.1% TFA

▶▶ Sample preparation

1. Deposit 1–5 μL sample solution onto a sample spot.

The sample should be acidified (that is, contain 0.1% TFA) before spotting.

Samples must not contain any organic solvent. The presence of organic solvents will reduce the efficiency of peptide adsorption to the HCCA matrix thin layer.

2. Incubate the sample on the matrix spot for 3 minutes and reaspirate the sample liquid.
3. Wash all sample and external calibrant spots by depositing 1 μL cold Washing Buffer and reaspirating the liquid after 1–2 seconds.
 - a. Alternatively, the whole target can be washed by dipping it for 3–5 seconds into a beaker (600 mL capacity) filled with wash buffer at 4–8°C. Avoid moving the target while it is submerged in the washing buffer. After removal, allow residual washing buffer to dry completely before proceeding.

3.5 PAC Targets, Dried Sample Method

This preparation protocol enables highly sensitive measurements of peptides (for example, protein digests from 2D gel spots) in both MS and LIFT-MS/MS mode.

As for thin layer preparations in general, the MS/MS capacity of PAC target spots is limited due to the low amount of matrix available.

However, PAC targets prepared according to this protocol commonly enable acquisition of up to 10 LIFT-MS/MS spectra per spot, which is sufficient for routine protein identification workflows using 2D gel digests or for LC-MALDI analyses of low- to medium-complexity samples.

Sample type

- Peptides, protein digests

Chemicals and materials required

- Washing Buffer — 10 mM ammonium phosphate, monobasic (for example, Sigma-Aldrich A1645) in 0.1% TFA

▶▶ Sample preparation

1. Deposit 0.5–1 μL sample solution onto a sample spot.

The sample should be acidified (that is, contain 0.1% TFA) before spotting.

To prevent prespotted matrix from dissolving, samples should have an acetonitrile concentration $\leq 30\%$ (for 0.5 μL sample spots) or $\leq 15\%$ (for 1 μL sample spots). The acetonitrile concentration of samples can be reduced by depositing a droplet of water onto the target before depositing samples. For LC fractions, a water sheath flow can be used to dilute samples.

2. Allow the sample to dry at room temperature.

The drying process can be speeded up using a gentle stream of warm air from a hairdryer.

3. Wash all sample and external calibrant spots by depositing 1 μL cold Washing Buffer and reaspirating the liquid after 1–2 seconds.

- a. Alternatively, the whole target can be washed by dipping it for 3–5 seconds into a beaker (600 mL capacity) filled with wash buffer at 4–8°C. Avoid moving the target while it is submerged in the washing buffer. After removal, allow residual washing buffer to dry completely before proceeding.

3.6 2,5-DHB Dried Droplet, Ground Steel Targets

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% H₃PO₄.

▶▶ 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 and allow to dry.

3.7 2,5-DHB Dried Droplet, AnchorChip Targets

Note This protocol is also suitable for SDHB.

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

▶▶ Sample preparation

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

3.8 2,5-DHAP Dried Droplet, Ground Steel Targets

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 ($\text{C}_6\text{H}_8\text{O}_7 \cdot 2\text{NH}_3$).

▶▶ 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 and allow to dry.

3.9 SA Double Layer, Ground Steel Targets

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

3.10 SDHB Dried Droplet, Ground Steel Targets

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 μ L of the matrix/analyte mixture onto the MALDI target and allow to dry.

3.11 1,5-DAN Dried Droplet, Ground Steel Targets

Sample type

- Top-down sequencing of intact proteins (ISD; T³)

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: DANGER (H: 351, 410; P: 273, 281, 501)

▶▶ Sample preparation

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

3.12 3-HPA Dried Droplet, Ground Steel Targets

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 solution of diammonium hydrogen citrate ($C_6H_8O_7 \cdot 2NH_3$).

▶▶ Sample preparation

1. Deposit 0.5 μ L of the matrix solution onto each target position and allow to dry.
2. Deposit 0.5 μ L of the sample solution onto each matrix spot and allow to dry.

3.13 3-HPA Dried Droplet, SmallAnchor Targets

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 ($C_6H_8O_7 \cdot 2NH_3$) in water.

▶▶ Sample preparation

1. Deposit 1 μ L of the matrix solution onto each target position and allow to dry.
2. Deposit 1 μ L of the sample solution onto each matrix spot and allow to dry.

4 Ordering Information

Product	Part Number
MALDI targets	
MSP 96 target ground steel BC	# 280799
MSP 96 target polished steel BC	# 280800
MTP 384 target plate ground steel BC	# 280784
MTP 384 target plate polished steel BC	# 280781
MSP AnchorChip 96 BC	# 280823
MTP AnchorChip 384 BC	# 280790
MTP SmallAnchor 384 BC	# 280792
MTP AnchorChip 1536 BC	# 280787
PAC II 384 HCCA, 10 targets with 384 sample and 96 calibration spots	# 255682
PAC II 96 HCCA, 10 targets with 96 sample and 24 calibration spots	# 255683
MALDI matrices	
HCCA; α -Cyano-4-hydroxycinnamic acid, 1 g	# 201344
2,5-DHB; 2,5-Dihydroxybenzoic acid, 1 g	# 201346
2,5-DHAP; 2,5-Dihydroxyacetophenone, 1 g	# 231829
SA; Sinapinic acid, 1 g	# 201345
sDHB, 5 g	# 209813
3-HPA; 3-Hydroxypicolinic acid, 1 g	# 201224
MALDI calibration standard	
Peptide Calibration Standard II	# 222570

5 Manufacturer



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