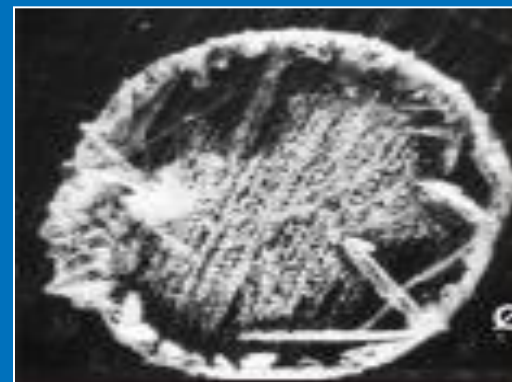
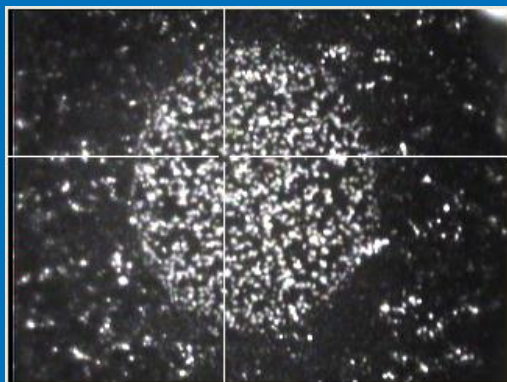


Bruker Daltonics

MALDI preparation protocols

Life science

(peptides, proteins, glycans, oligonucleotides)



Used Nomenclature



Target types:	Ground Steel	<i>Bruker Part no. #8280784</i>
	AnchorChip Standard (800µm)	<i>Bruker Part no. #8280790 (384 format)</i> <i>Bruker Part no. #8280787 (1536 format)</i>
	BigAnchor (2000µm)	<i>Bruker Part no. #8280788</i>
	AnchorChip Small (400µm)	<i>Bruker Part no. #8280792</i>
	Pre-spotted AnchorChip (PAC)	
Matrices & additives:	HCCA	α -Cyano-4-hydroxy cinnamic acid (<i>Bruker Part-No. #8201344</i>)
	2,5-DHB	2,5-Dihydroxy benzoic acid (<i>Bruker Part no. #8201346</i>)
	sDHB	90:10 mixture of 2,5-DHB and 2-Hydroxy-5-methoxy benzoic acid (<i>Bruker Part no. #8209813</i>)
	2,5-DHAP	2,5-Dihydroxy acetophenone (<i>Bruker Part No. #8231829</i>)
	SA	Trans-3,5-dimethoxy-4-hydroxy cinnamic acid (<i>Bruker Part No. #8201345</i>)
	1,5-DAN	1,5-Diaminonaphthalene
	3-HPA	3-Hydroxy picolinic acid (<i>Bruker Part No. #8201224</i>)
	DAC	Diammonium hydrogen citrate
	NH ₄ H ₂ PO ₄	Ammoniumphosphate, monobasic
	TFA	Trifluoro acetic acid
Solvents:	ACN	Acetonitrile
	TA30	30:70 (v/v) Acetonitrile : TFA 0.1% in water
	TA50	50:50 (v/v) Acetonitrile : TFA 0.1% in water
	TA85	85:15 (v/v) Acetonitrile : TFA 0.1% in water
	TA90	90:10 (v/v) Acetonitrile : TFA 0.1% in water
	TA95	95:5 (v/v) Acetonitrile : TFA 0.1% in water
	EtOH	Ethanol
H ₂ O	Water	

Protocol overview



Matrix	Protocol	Target type	Page
HCCA	Dried droplet	Ground Steel	6
	Dried droplet	AnchorChip (800µm)	7
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Types of target plates – when to use what



Ground steel:

Standard plate for fast, simple and robust MALDI preparation of virtually all types of samples.

AnchorChip (800µm):

Preferred type of plate to be used in all kinds of high throughput MALDI measurements performed in unattended, fully automatic mode, e.g. MALDI peptide mapping of 2D gel digests, LC-MALDI analyses of complex peptide mixtures etc.

AnchorChip Standard plates allow for 100% precise spot alignment. This is due to the hydrophilic properties of the „anchors“ which cause a self-centering effect upon the process of drying down matrix preparations. Highly precise spot alignment achieved on AnchorChip plates is of great benefit to the efficiency of MALDI signal generation during automatic runs, resulting in greater S/N at reduced acquisition time.

In general, AnchorChip plates offer improved sensitivity due to the focussing effect achieved upon drying down sample preparations.

BigAnchor (2000µm):

BigAnchor plates offer a wider spot diameter (2000µm). BigAnchor MALDI plates are intended for use with MALDI matrices that are difficult to prepare on the narrow spots featured by the 800µm Anchorchip plates (f.e. 2,5-DHAP matrix).

AnchorChip Small (400µm):

Dedicated to the preparation of oligonucleotides and similar samples using 3-HPA as a matrix.

Pre-spotted AnchorChip (PAC): +++ no longer available +++

These disposable plates allow for most convenient, fast and highly sensitive preparation of peptides or further samples that require HCCA as a MALDI matrix. According to their nature as a disposable product, PAC targets offer 100% safety with regard to memory effects. As the pre-deposited HCCA thinlayer spots offer limited matrix capacity, these plates are ideally suited for low to medium complexity samples, e.g. in-gel digests of proteins from 2D gels and similar.

Target cleaning



Target type: Ground steel / AnchorChip / BigAnchor

Solvent A: 2-Propanol

Solvent B: Water

Solvent C: TA30

Cleaning protocol:

- Wipe off old preparations using tissue wetted with **solvent A** and **solvent B**, subsequently.
- Sonicate the target plate for 10min using **solvent A**.
- Sonicate the target plate for another 10min using **solvent C**.
- Dry the target plate using high purity nitrogen or air. Don't wipe the target front side of the cleaned plate. If pure gases are not available, let dry the plate under room conditions.

HCCA, dried droplet

Peptides; Protein digests



Target type: Ground steel

Matrix Solution: HCCA saturated in TA30

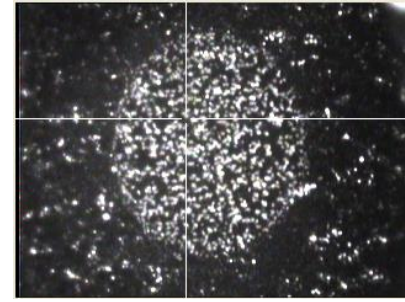
Sample Solvent: 0.1% TFA or TA30

Preparation protocol:

Pre-mix equal volumes (for example 2µl each) of sample solution and matrix solution. Apply 0.5 µl of this mixture onto a ground steel target.

HCCA, dried droplet

Peptides; Protein digests



Target type: AnchorChip (800 μ m)

Matrix Solution:

1.4mg/ml HCCA, dissolved in a solvent mixture containing 85% ACN, 15% H₂O, 0.1% TFA and 1mM NH₄H₂PO₄

Sample Solvent: 0.1% TFA or TA30

Preparation protocol:

Spot 0.5 – 1 μ l of the sample solution onto the target. Wait until the spotted sample aliquot has dried down. Subsequently, add 0.5 μ l of the matrix solution. Let the preparation dry down at room temperature.

Note:

Make sure the sample does not contain alkaline salts, surfactants or other contaminants that are known to interfere with MALDI. In case of presence of such contaminants, clean-up the sample in advance to MALDI analysis using magnetic beads, ZipTips, dialysis membranes or similar devices.

Preparation of external calibrant spots:

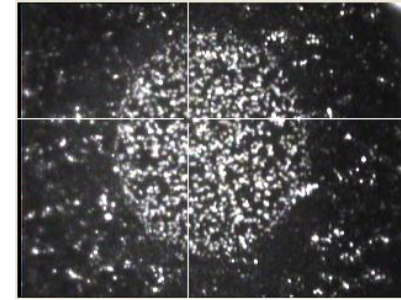
Dissolve the “Peptide Calibration Standard II” (Bruker part number #8222570) in 125 μ l of TA30. Pre-mix this calibrant solution with the HCCA matrix solution in a ratio 1:200. Deposit 0.5 μ l of this mixture onto the next neighboring calibrant spot position on the AnchorChip target plate.

HCCA, dried droplet

nanoLC-MALDI analysis of peptide mixtures

(using typical nanoLC flow rates of 300nl/min)

Target type: AnchorChip (800µm)



1) Spotting method for nanoLC fractions:

800µl of the HCCA matrix solution to be used consist of the following components:

- 748µl **TA95**
- 36µl of HCCA stock solution (= HCCA saturated in TA90)
- 8µl TFA 10% in water
- 8µl of 100mM $\text{NH}_4\text{H}_2\text{PO}_4$ dissolved in water

Using the matrix solution as described above, the syringe pump supplying the matrix should be set to a flow rate of 100µl/h when spotting 15s fractions, and of 150µl/h when spotting 10s fractions. This corresponds, in both cases, to an absolute amount of matrix solution per spot of approx. 420nl.

2) Spotting method for external calibrants:

For preparation of external calibrant spots, a matrix solution is used that contains a somewhat higher percentage of water.

800µl of the HCCA matrix solution used for the calibrant spots consist of:

- 748µl **TA85**
- 36µl of HCCA stock solution (= HCCA saturated in TA90)
- 8µl TFA 10% in water
- 8µl of 100mM $\text{NH}_4\text{H}_2\text{PO}_4$ dissolved in water

Dissolve Bruker's „Peptide Calibration Standard II“ (Bruker part number #8222570) in 125µl of TA30 (= 30% ACN, 70% water, 0.1%TFA). Mix 300µl of the matrix solution prepared for spotting the calibrants with 1.5µl of the Peptide Calibration Standard II solution described above. Spot 420nl of this mixture on each of the calibrant positions.

HCCA, thinlayer

Peptides; Protein digests



Target type: Pre-spotted AnchorChip (PAC384, PAC96)

Protocol A) “Dried sample” method:

Considerations:

In general, samples should be acidified prior to spotting, i.e. should contain 0.1% TFA.

When using the “Dried sample” preparation method, the **sample solvent may not exceed a critical maximum percentage of organic solvent**. Excessive organic solvent would lead to undesired dissolution of the HCCA matrix spots. When using ACN as the organic component in the sample solvent, the following percentages of ACN represent the maximum that the HCCA will tolerate when a given sample volume is spotted:

Maximum 30% ACN @ a spotted sample volume of 0.5µl

Maximum 15% ACN @ a spotted sample volume of 1.0µl

Spotting protocol:

Spot a sample aliquot (0.5 - 1µl are recommended) onto the pre-spotted HCCA thin layer.

Let the sample droplet dry down completely at room temperature. A gentle air stream at elevated temperature (hair dryer) may be applied to accelerate the drying process. When the sample droplet has dried down completely, wash on-target the prepared spots applying 1µl aliquots of a cold buffer containing 10mM $\text{NH}_4\text{H}_2\text{PO}_4$ dissolved in 0.1%TFA in water. Remove the wash buffer from the spot after 1-2 seconds of incubation time.

Note:

The external calibrant spots (which are readily available on the PAC target plates) should be washed on-target using exactly the same protocol as described above.

Characteristics of the “Dried sample” preparation method:

This preparation protocol enables highly sensitive measurements of peptides (e.g. 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 limited amount of matrix available. However, PAC targets prepared according to the “Dried sample” protocol commonly provide sufficient capacity for the acquisition of up to 10 LIFT-MS/MS spectra per spot, which is adequate for routine protein identification workflows for 2D gel digests as well as for LC-MALDI analyses of low to medium complexity samples.



HCCA, thinlayer

Peptides; Protein digests



Target type: Pre-spotted AnchorChip (PAC384, PAC96)

Protocol B) "Affinity" method:

Considerations:

In general, samples should be acidified prior to spotting, i.e. should contain 0.1% TFA.

When using the "Affinity" preparation method, **samples must not contain any organic solvent**. This would reduce the efficiency of peptide adsorption to the HCCA matrix thin layer, which is highly undesirable since this interaction is the basic principle of the "Affinity" preparation technique.

Spotting protocol:

Deposit a sample aliquot (1-5 μ l are recommended) onto the pre-spotted HCCA thin layer. Incubate the sample droplet on the spot for 3 minutes, then remove the sample droplet from the spot and wash the prepared spots on-target applying 1 μ l aliquots of a cold buffer containing 10mM $\text{NH}_4\text{H}_2\text{PO}_4$ dissolved in 0.1%TFA in water.

Note:

The external calibrant spots (which are readily available on the PAC target plates) should be washed on-target using exactly the same protocol as described above.

Characteristics of the "Affinity" preparation method:

This preparation method is based on immobilization of peptides on the pre-coated HCCA matrix spots by means of the adsorption equilibrium which is maintained between the deposited peptide sample solution and the outer crystal surface of the HCCA matrix thin layer during the incubation step. The "Affinity" protocol yields MS spectra of high signal to noise for peptide digests.

As the "affinity" method is driven by adsorption equilibria, highly hydrophilic peptides and other peptides being adsorbed less efficiently on the HCCA matrix crystal surface may be detected in the resulting MALDI spectra at lowered relative intensities or may even not be detected at all.

Preparations obtained from the "Affinity" method, in general, offer a lower spot capacity for MS/MS acquisitions compared to the previously described "Dried sample" technique.

However, the merits of the "Affinity" method lay in its somewhat higher tolerance against contaminants that are typically contained in digest samples (i.e. buffer salts, surfactants etc.), which may reduce the need for additional sample clean-up steps (zipTip or similar) prior to MALDI spotting.



2,5-DHB, dried droplet

**Peptides; Phosphoprotein digests; Glycoprotein digests;
Intact proteins; Glycans**

Target type: Ground steel

Matrix Solution: 20mg/ml 2,5-DHB in TA30.

Optional additives: In case of phosphopeptide analyses: 1% H_3PO_4 .
In case of glycan analyses: 1mM NaCl.

Sample Solvent: 0.1% TFA or TA30; in case of glycans: water

Preparation protocol:

Pre-mix equal volumes (for example 2 μl each) of sample solution and matrix solution. Apply 0.5 μl of this mixture onto a ground steel target.

2,5-DHB, dried droplet

Peptides; Glycans; Glycoprotein Digests

Target type: AnchorChip (800 μ m)

Matrix Solution: 10mg/ml 2,5-DHB in TA30

Optional additives: In case of glycan analyses: 1mM NaCl

Sample Solution: 0.1% TFA or TA30; in case of glycans: water

Preparation protocol:

Prepare 0.5 μ l of matrix solution on a 800 μ m AnchorChip target. Let the matrix dry down completely. Spot 0.5-1 μ l of sample solution on top of the dried matrix preparation and let it dry at room temperature.

2,5-DHAP, dried droplet

Intact proteins

Target type: Ground steel; BigAnchor (2000 μ m)

Matrix Solution:

Dissolve 7.6mg 2,5-DHAP in 375 μ l EtOH.

Add 125 μ l of a solution containing 18mg/ml DAC dissolved in water.

Sample Solvent: 0.1% TFA

Preparation protocol:

Mix 2 μ l of the sample solution with 2 μ l of a 2% TFA solution. Add 2 μ l of the matrix solution. Pipette up and down this ternary mixture until the crystallization starts. Spot 0.5 μ l of the crystal suspension onto the target.

SA, double layer

Intact proteins

Target type: Ground steel

Matrix Solution I: SA saturated in EtOH

Matrix Solution II: SA saturated in TA30

Sample Solvent: 0.1% TFA

Preparation protocol:

Prepare a matrix thinlayer on a ground steel target using matrix solution I.

Pre-mix equal volumes (for example 2 μ l each) of sample solution and matrix solution II. Apply 0.5 μ l of this mixture on top of the matrix thinlayer prepared in advance.

sDHB, dried droplet

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

Target type: Ground steel

Matrix Solution: 50mg/ml sDHB in TA50

Sample Solvent: 0.1% TFA or TA30

Preparation protocol:

Pre-mix equal volumes (for example 2 μ l each) of sample solution and matrix solution. Apply 0.5 μ l of this mixture onto a ground steel target.

sDHB, dried droplet

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

Target type: BigAnchor (2000 μ m)

Matrix Solution: 25mg/ml sDHB in TA50

Sample Solvent: 0.1% TFA or TA30

Preparation protocol:

First, spot a μ l aliquot of the sample solution and let it dry down. Subsequently, add 1 μ l of the matrix solution and let it dry at room conditions.

sDHB, dried droplet

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

Target type: AnchorChip (800 μ m)

Matrix Solution: 10mg/ml sDHB in TA50

Sample Solvent: 0.1% TFA or TA30

Preparation protocol:

First, spot 0.5-1 μ l aliquot of the sample solution and let it dry down. Subsequently, add 0.5 μ l of the matrix solution and let it dry at room conditions.

1,5-DAN, dried droplet

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

Target type: **Ground steel**

Matrix Solution: 1,5-DAN saturated in TA50.

Note:

Prepare matrix solution freshly prior to usage (to avoid degradation of matrix solution)!

Note:

1,5-DAN is a potentially cancerogenic substance (R40-50/53)! Handle with care!

Sample Solvent: 0.1% TFA or TA30

Preparation protocol:

Pre-mix sample solution and matrix solution in a ratio of 1:2 (v:v), for example 2 μ l of sample solution and 4 μ l of matrix solution. Apply 0.5 μ l of this mixture onto a ground steel target.

3-HPA, dried droplet

Oligonucleotides

Target type: Ground steel

Matrix Solution:

3-HPA saturated in H₂O:ACN 50:50 (v:v), containing 10mg/ml DAC

Sample Solvent: water

Preparation protocol:

Apply 0.5µl of the matrix solution on the target. Let the matrix dry down at room temperature. Add 0.5µl of the sample solution on top of the dried matrix preparation spot. Let the preparation dry down at room temperature.

3-HPA, dried droplet

Oligonucleotides

Target type: AnchorChip (800 μ m)

Matrix Solution:

Solvent: H₂O:ACN 50:50 (v:v), containing 10mg/ml
DAC

HPA concentration: half-saturated

Sample Solvent: water

Preparation protocol:

Apply 0.5 μ l of the matrix solution on the target. Let the matrix dry down at room temperature. Add 0.5 μ l of the sample solution on top of the dried matrix preparation spot. Let the preparation dry down at room temperature.

3-HPA, dried droplet

Oligonucleotides

Target type: AnchorChip Small (400 μ m)

Matrix Solution:

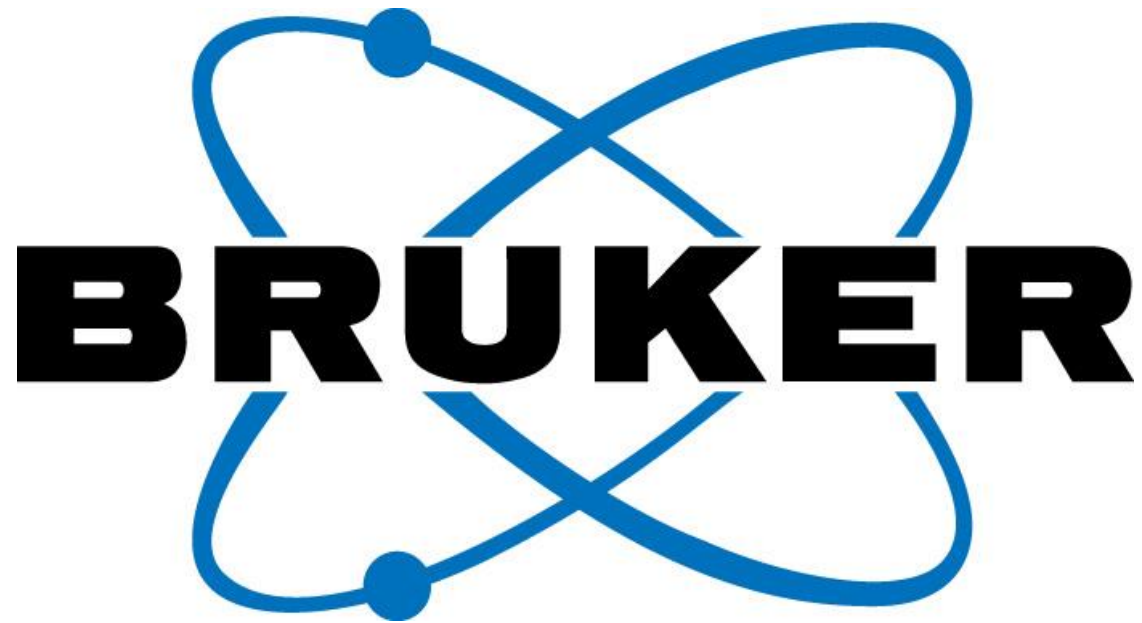
10mg/ml 3-HPA in water, containing 1mg/ml diammonium hydrogen citrate

Sample Solvent: water

Preparation protocol:

Apply 1 μ l of matrix solution onto a 400 μ m AnchorChip target and let dry.

Add 1 μ l oligonucleotide solution on top of the dried matrix. Let the preparation dry at room temperature.



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