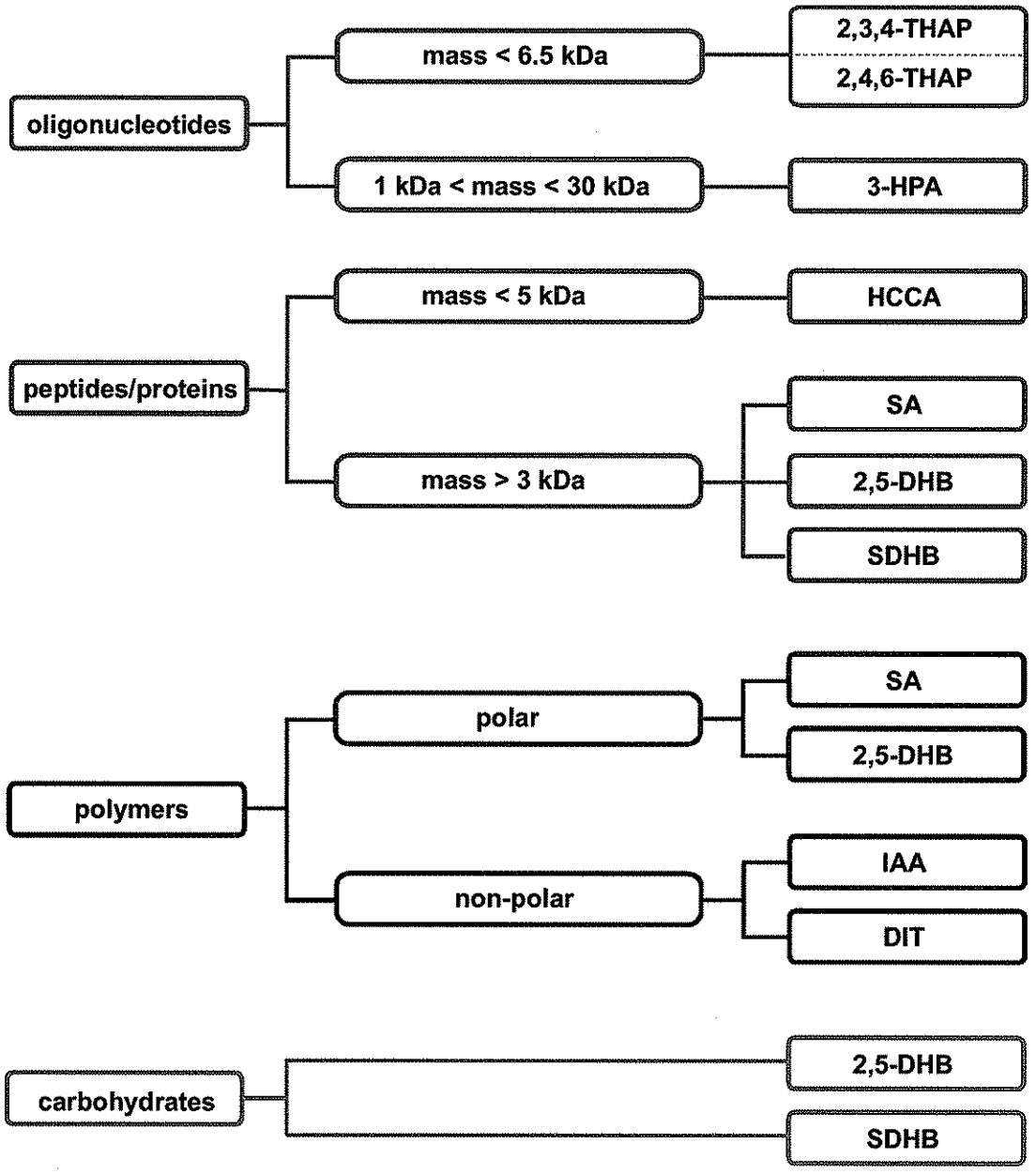
Matrix Guide to Sample Preparation



List of Abbreviations

HCCA	α-Cyano-4-hydroxycinnamic acid
2,5-DHB	2,5-Dihydroxybenzoic acid
2,3,4-THAP	2,3,4-Trihydroxyacetophenone
2,4,6-THAP	2,4,6-Trihydroxyacetophenone
DIT	Dithranol
SA	Sinapinic acid
SDHB	Super DHB
IAA	trans-Indole-3-acrylic acid
3-HPA	3-Hydroxypicolinic acid
CNME	α-Cyano-4-hydroxycinnamic acid methyl ester
ACN	Acetonitrile
TFA	Trifluoro acetic acid

Sample Preparation - Examples

In the following sections sample preparation protocols are described for different compounds classes, which essentially follow published procedures. Unless otherwise stated, obtain spectra always in positive ion mode.

Oligonucleotides

For the analysis of mixed oligonucleotides up to 30 kDa, 3-HPA has proved useful as a matrix material. Nucleotides are relative labile molecules giving rise to a large extent of metastable fragmentation. Impurities like salts may decreased the sensitivity. Therefore, complete removal of cations like sodium and potassium is mandatory to successful analyzes.

We recommend the following preparation protocols:

1. Standard preparation for steel targets

Matrix: *3-HPA/Diammoniumhydrogencitrate*

Prepare a saturated solution of 3-HPA in 50 % acetonitrile by adding 3-HPA to 50 % acetonitrile/water and vortexing for 1 min. A sediment must remain at the bottom of the tube. Add 100 µl of diammoniumhydrogencitrate solution (100 g/l) to 900 µl of the saturated 3-HPA solution (final conc. 10 g/l).
We recommend Bruker ultrapure 3-HPA (no treatment with cation exchange resin necessary). Matrices of minor quality may be improved by adding cation exchange beads (Biorad AG 50W-X8) loaded with ammonium ions.

Target preparation:

Mix 1 µl of analyte (e.g. pure oligonucleotide solution) with 1 µl of 3-HPA matrix solution (see above). Apply 1µl of the mixture to a sample position of the MALDI target and allow to dry.

alternatively:

Add 1 µl 3-HPA matrix solution to the MALDI target, let dry. Apply 1µl of analyte (e.g. pure oligonucleotide solution) to the crystals and allow to dry again. This procedure will lead to preferred incorporation of the analyte into the surface of the matrix crystals and thereby increase signal intensity in MALDI measurement.

Comments:

- Usually 2pmol of a pure 20-30mer will result in good signal intensities.
- The MALDI target should be cleaned well, e.g. by treatment with 50 % acetonitrile in a ultrasonic devive followed by polishing with methanol.
- Contamination of the analyte, e.g. with alkali ions or detergents, will lead to adduct formation and may prevent successful MALDI measurement at all.

2. Standard preparation for AnchorChips (400 µm)

Matrix: *3-HPA/Diammoniumhydrogencitrate*

Prepare a solution of 10 g/l 3-HPA, 1 g/l diammoniumhydrogencitrate.
We recommend Bruker ultrapure 3-HPA (no treatment with cation exchange resins necessary).

Target preparation:

Apply 1 µl of 3-HPA matrix solution (see above) to a 400 µm anchor of an AnchorChip. Allow to dry. The matrix will build a small crystal at the anchor position. Add 1 µl of the analyte solution (e.g. pure oligonucleotide solution) to the preloaded anchor. Allow to dry again.

Comments:

- Usually 0.5 pmol of a pure 20-30mer will result in good signal intensities.
- The AnchorChip should be cleaned well.
  The following protocol is recommended:

  Rinse the AnchorChip with water to remove old matrix
  Clean with isopropanol
  Clean with methanol
  15 min in ultrasonic bath with preheated (about 60 °C) ultrapure water.

- Contamination of the analyte, e.g. with alkali ions or detergents, will lead to adduct formation and may prevent successful MALDI measurement at all. The concentrated sample is more susceptible to such adduct formation than macropreparations.

Reference for oligonucleotide analyzes

Eckhard Nordhoff, Martin Schürenberg, Gabriela Thiele, Christine Lübbert, Klaus-Dieter Kloeppel, Dorothea Theiss, Hans Lehrach, Johan Gobom: Sample Preparation Protocols for MALDI-MS of Peptides and Oligonucleotides Using Prestructured Sample Supports; International Journal of Mass Spectrometry 226 (2003) 163-180.

Peptides and Proteins

The standard matrices are HCCA, SA and 2,5-DHB. HCCA is mainly used for peptides up to ca. 5 kDa, since fragmentation becomes exceedingly strong in larger proteins. SA gives good results with peptides and proteins larger than ca. 2 kDa. 2,5-DHB is also recommended for glycoproteins.

Peptides

To get started with robust HCCA Dried Droplet or Thin Layer Preparation on metal target.

Dried Droplet Method

Standard sample preparation technique on metal targets:

- *Matrix solution:* HCCA saturated in TA (ACN : 0.1 % TFA, 1:2)
- *Sample solution:* mix 0.5-1.0 analyte solution with 0.5 µl matrix solution on target

10-100 fold increased sensitivity on Bruker AnchorChip targets. For more information, please see dedicated protocols in AnchorChip manual.

Real life in gel digest samples often require purification (e.g. desalting). In this case use µC18-ZipTip (Millipore) or on-target purification (HCCA Thin Layer on metal target or HCCA affinity AnchorChip preparation.

HCCA - Thin Layer Method

- Apply 0.5 µl HCCA (saturated in acetone) onto metal target (-> thin matrix layer)
- Alternatively you can use a HCCA/nitrocellulose mixture:
    - mix 4 parts HCCA (saturated in acetone) with 1 part NC (20 g/l nitro-cellulose in acetone/2-propanol 1:1) and apply 0.5 µl of this mixture onto metal target
- Deposit 0.5 µl acidified analyte solution (pH<6) onto thin matrix layer. If the analyte solution has pH>6 then the thin matrix layer would be redissolved!
- Let the sample dry (in case of air oxidation of the analyte use vacuum-drying at 100 mbar)
- Wash 2-3 times with 5-10 µl cooled 0.1 % TFA

Proteins

Standard preparation protocols for proteins (>3 kDa)

A: SA Dried Droplet Method

- Mix 0.5 µl analyte solution with 0.5 µl SA (saturated in ACN : 0.1 % TFA, 1:2) on target

B: SA Double Layer Method (enhanced sensitivity)

- Prepare a thin layer from SA saturated solution in ethanol
- Dilute sample to a few pmol/µl with 0.1 % TFA
- Mix equal volumes of sample with SA (saturated in ACN : 0.1 % TFA, 1:2)
- Apply 0.5 µl onto the SA layer

C: 2,5-DHB (or SDHB) Dried Droplet Method

- Mix 0.5 µl analyte solution with 0.5 µl 2,5-DHB (10 g/l in ACN : 0.1 % TFA , 1:2) on target
- Using SDHB instead of 2,5-DHB decrease fragmentation of large proteins (>50 kDa)

Reference for peptide and protein analyzes

Eckhard Nordhoff, Martin Schürenberg, Gabriela Thiele, Christine Lübbert, Klaus-Dieter Kloeppel, Dorothea Theiss, Hans Lehrach, Johan Gobom: Sample Preparation Protocols for MALDI-MS of Peptides and Oligonucleotides Using Prestructured Sample Supports; International Journal of Mass Spectrometry 226 (2003) 163-180.

Martin Kussmann, Peter Roepstorff: Sample Preparation Techniques for Peptides and Proteins Analyzed by MALDI-MS; from: Methods in Molecular Biology, vol. 146: Protein and Peptide Analysis: New Mass Spectroscopic Applications; Edited by J.R. Chapman, Humana Press Inc., Totowa, NY.

Polymers

There are at least hundreds of classes of polymers that are different with respect to their molecular weight (ca. 5-1000 kDa), chemical and physical properties. Even the change of an end-group might completely change the ionization probabilities of a polymer.

This large variety of structures prevents general protocols that are applicable to any kind of polymer. A possible classification of practical importance with respect to MALDI analysis is their solubility in aqueous solution. For **water soluble polymers**, 2,5-DHB is usually a good matrix while for **polymers soluble in organic solvents** you can use Dithranol, all-trans-Retinoic acid, 2,5-DHB, 5-chlorosalicylic acid and IAA. **Polymers that are insoluble** at all, can be prepared in solid state[1]. Care should be employed for finding a good solvent for the polymer. If a polymer is insoluble in water, try to find a fully organic solvent system to avoid precipitation during the sample preparation (e.g. tetrahydrofuran, hexafluor-isopropanol, acetone, methanol or chloroform). Most polymers need a cation to form charged species. In order not to get a mixture of ion series and to support the ionization very often a slat is added (mainly TFA-salts because of their good solubility in organic solvents).

A very good database for preparation protocols can be found on the webpage of the National Institute of Standards and Technology:

http://polymers.msel.nist.gov/maldirecipes/index.cfm

Reference for polymer analyzes

[1] *Trimpin, S., A. Rouhanipour, R. Az, H. J. Räder, and K. Müllen:*
New Aspects in Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry: A Universal Solvent-Free Sample Preparation.
Rapid Communications in Mass Spectroscopy. **15**, 1364-73 (2001)

Carbohydrates

Also for carbohydrates, 2,5-DHB is the preferred matrix. Positive mode spectra yield predominantly the $[M+Na]^+$ and $[M+K]^+$ cationized molecular species (mass difference between them is 16!).

- **Matrix:** make 20 mg/ml solution of 2,5-DHB in 20-80 % ethanol or water.
- Make a 10 pmol/µl solution of oligosaccharide in 20-80 % ethanol or water.
- Mix sample and matrix solution in a ration 1:1.
- Apply 0.5 µl onto sample target.
- Use a gentle stream of cold air from a hairdryer to assist sample drying.

For more information, please visit our worldwide web sites on the internet or call us by phone.

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Product Information

Peptide/Protein Matrix Kit

Purified matrix substances for matrix-assisted laser desorption and ionization time-of-flight mass spectrometers (MALDI-TOF MS)

#205931 **Version 01-11-02**

Product description

Contents: The kit contains 3 matrix substances (**2,5-Dihydroxybenzoic acid**, **Sinapinic acid** and **α-Cyano-4-hydroxycinnamic acid**) in 3 tubes with 10 mg for each matrix.

Storage: The matrix substances are shipped at ambient temperature. We recommend to store the substances on arrival in the darkness at 2-8 °C (36-46 °F).

Risk and safety information

2,5-Dihydroxybenzoic acid

R- & S-Phrases: R 22-36/37/38 ▪ S 22-36/37/38

Harmful

Harmful if swallowed. Irritating to eyes, respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. Wear suitable protective clothing and gloves.

Sinapinic acid

R- & S-Phrases: R 36/37/38 ▪ S 26-28-37/39

Irritant

Irritating to eyes, respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. Wear suitable gloves and eye/face protection.

α-Cyano-4-hydroxycinnamic acid

R- & S-Phrases: R 36/37/38 ▪ S 24/25-26-28-37/38

Harmful

Harmful by inhalation, in contact with skin and if swallowed. Irritating to eyes, respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of Water. Wear suitable protective clothing and gloves.

For more information, please read the Material Safety Data Sheets.