Second Edition April 2006

Mass·Spec·Turbo Chip Handbook

Mass·Spec·Turbo Peptide Chips

Mass·Spec·Turbo Protein Chips

For high-throughput MALDI-MS analysis of peptides and proteins

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Contents

Kit Contents

Mass·Spec·Turbo 192/625/1600 Peptide Chips Type 1 For Applied Biosystems 4700 instruments	**Cat. nos. 49000/ 49002/49004**
Mass·Spec·Turbo 192/625/1600 Peptide Chips	2
Finishing Solution	2 x 100 ml
Handbook	1

Mass·Spec·Turbo 192/625/1600 Peptide Chips Type 2 For Applied Biosystems 4700 instruments	**Cat. nos. 49001/ 49003/49005**
Mass·Spec·Turbo 192/625/1600 Peptide Chips	2
Finishing Solution	2 x 100 ml
Handbook	1

Mass·Spec·Turbo 192 Protein Chips Type 1 For Applied Biosystems 4700 instruments	**Cat. no. 49100**
Mass·Spec·Turbo 192 Protein Chips	2
Finishing Solution	2 x 100 ml
Handbook	1

Mass·Spec·Turbo 192 Protein Chips Type 2 For Applied Biosystems QSTAR and Voyager instruments	**Cat. no. 49101**
Mass·Spec·Turbo 192 Protein Chips	2
Finishing Solution	2 x 100 ml
Handbook	1

Storage

Mass·Spec·Turbo Chips should be stored in their original packaging in dry conditions at room temperature (15–25°C) and protected from dust and light. Do not expose Mass·Spec·Turbo Chips to direct sunlight. A standard laboratory dessicator is ideal for storage. Chips can be stored under these conditions for up to six months, without any decrease in performance.

When stored under these conditions, test spots with peptide standards, as well as user samples, did not exhibit any decrease in sensitivity up to six months after sample preparation.

Finishing Solution should be stored at 2–8°C upon arrival, and once opened should be used within 4–6 weeks.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/ts/msds.asp where you can find, view, and print the MSDS for each QIAGEN® kit and kit component.

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany, Tel: +49-6131-19240

Quality Control

In accordance with QIAGEN's ISO-certified Total Quality Management System, each lot of Mass·Spec·Turbo Chip Kits is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

Mass·Spec·Turbo Chip Kits are developed, designed, and sold for research purposes only. It is not to be used for human diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in this text.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see inside back cover).

Technical Assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN products. If you have any questions or experience any difficulties regarding Mass·Spec·Turbo Chip Kits or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information please call one of the QIAGEN Technical Service Departments or local distributors (see inside back cover).

Introduction

Mass·Spec·Turbo Chips offer researchers an elegant solution to some of the sample preparation problems often associated with matrix assisted laser desorption/ionization (MALDI) mass spectrometry (MS). The ready-to-use chips provide precisely defined and highly homogenous pre-deposited matrix spots surrounded by an ultrahydrophobic surface. The ultrahydrophobicity of the area surrounding the spots delivers "on-chip" sample concentration and the fine structure of the vacuum-sublimated matrix crystals enables optimal incorporation of analyte, speeding and facilitating MALDI-MS analyses. The large number of spots on Mass·Spec·Turbo Chips enables comprehensive high-throughput analysis of liquid chromatographic (LC) separations. Fractions can be automatically applied to chips using a microfraction collector (e.g., Probot).

The MALDI experimental process

Matrix assisted laser desorption ionization (MALDI) mass spectrometry (MS) was developed in the late 1980s for mass spectral analysis of polypeptides, proteins, and oligonucleotides. The main advantage of MALDI-MS over conventional MS is that during the soft ionization process, little or no fragmentation takes place, greatly facilitating identification of analyte molecular ions, even within mixtures. The coupling of this technology with liquid chromatography separation (LC-MALDI-MS) has made it an extremely powerful tool in proteomics research.

Adding a low molecular weight organic matrix to a more massive analyte prevents molecular photodissociation of the sample ions induced by direct laser irradiation. The laser target is usually formed from a dilute solution of the analyte in a matrix of molecules that efficiently adsorb laser light. Such molecules include α-cyano-4-hydrocycinnamic acid (CHCA) or sinapinic acid (SA). The analyte/matrix mixture is applied to the target stage of a mass spectrometer and allowed to dry. Upon drying, a crystalline deposit is formed, comprising the matrix with evenly dispersed analyte molecules. The matrix is then irradiated by a short-duration pulse of laser light, which causes desorption (vaporization) of the analyte and matrix. Analytes are simultaneously protonated by the photo-excited matrix and can be separated in time when they are accelerated by a strong electric field under vacuum. After they hit a detector, their time-of-flight (TOF) is calculated. This time is a function of an ion's mass-to-charge (m/z) ratio, enabling an ion's mass to be derived from its TOF. Its sensitivity, speed, and ease of spectra analysis have made MALDI-TOF-MS a vital tool in proteomics and genomics projects.

Manual matrix and sample preparation

For a successful MALDI mass measurement, analytes must be incorporated into matrix crystals. This process can be disturbed by contaminants, such as detergents and salts. Matrices that allow a degree of "on-target" washing — which is useful for removing contaminants — are α-cyano-4-hydroxy-transcinnamic acid and sinapinic acid. Alternatively, water-soluble 2,5-dihydroxybenzoic acid may also be used, as this matrix excludes impurities during crystallization.

The simplest and most frequently used sample preparation method is the "dried-droplet" method. In this method the matrix, dissolved in an organic solvent (e.g., acetone or acetonitrile), is simply added to the sample on the sample stage. However, a common problem in the dried-droplet method is the aggregation of analyte/matrix crystals in a ring around the edge of the drop. Normally these crystals are inhomogeneous and irregularly distributed, which is the reason MALDI users often end up searching for "sweet spots" on their sample surfaces. Alternatively, a thin-layer of matrix material from a fast-evaporating solvent may be prepared first, onto which the sample is subsequently deposited. The latter sample preparation method often results in improved sensitivity and mass accuracy of peptides.

Because of differential mass discrimination/ion suppression effects, the sample preparation method can significantly affect results, especially when complex peptide mixtures are analyzed.

Mass·Spec·Turbo Chips — High-throughput and high sensitivity

Mass·Spec·Turbo Chips have been designed to overcome many of the limitations of manual sample preparation mentioned above. They are disposable devices, which comprise organic matrix spots (which are deposited by vacuum sublimation) surrounded by an ultrahydrophobic surface. The vacuum sublimation process delivers two major advantages over manual methods: matrix crystals are very fine and homogeneous compared to wet matrix preparations, and the matrix spots are very precisely defined with respect to size, shape, and position. Analyte samples are easily deposited on the matrix spots due to the properties of the ultrahydrophobic surface surrounding them, and as they dry, samples are concentrated in the upper layer of the matrix. Samples can be loaded manually (192-spot chips) or in an automated procedure using a microfraction collector (e.g., Probot) or spotting robot (Figure 1).

Easy Deposition of Samples

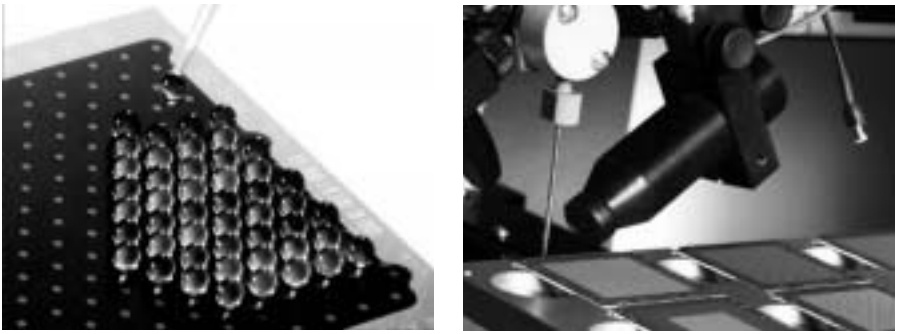


Figure 1 Samples are confined by the ultrahydrophobic surface surrounding the matrix spots.

Matrix Spots Contain Homogenous Crystals

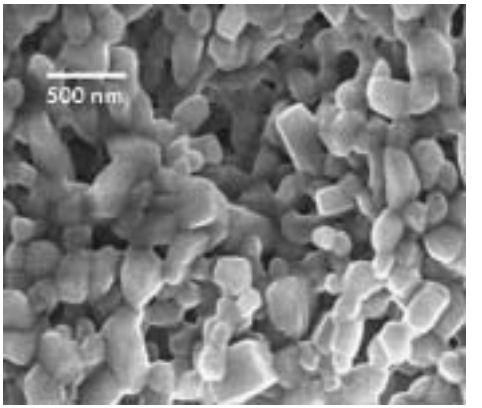
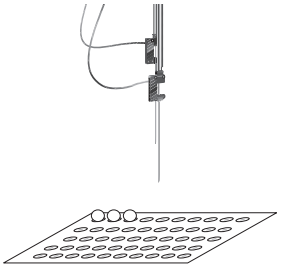


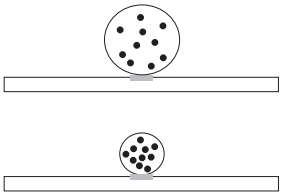
Figure 2 The vacuum sublimation procedure delivers fine, homogenous matrix crystals.

During sample preparation the fine and homogenous crystal structure of the matrix is retained. The average crystal size is around 300 nm (Figure 2). Using Mass·Spec·Turbo Chips, sample preparation is performed in two steps (see flowchart, page 10). After sample application the solvent is allowed to evaporate. During drying, the sample solvent partially dissolves the matrix spot and the analyte is incorporated into the matrix. Once drying is complete, the spot is treated with a finishing solution to enhance MALDI-MS performance.

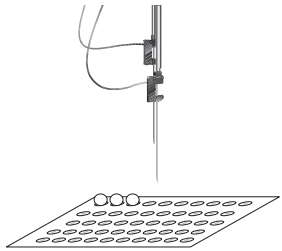
Mass·Spec·Turbo Procedure



Apply sample



Allow solvent to evaporate



Apply Finishing Solution

Analyze sample

Important factors during sample loading are liquid sample volume and solvent concentration. These factors greatly influence the final depth distribution of the analyte within the matrix spot and the resulting signals (Figure 3). For highest MS sensitivities, the analyte should be enriched at the surface of the spot. Therefore, sample volumes should be kept low (e.g., 0.1–2 µl for manual spotting). The organic proportion of solvents should also be kept low (e.g., 20% acetonitrile in water/0.1%TFA). Organic solvent concentrations above 50% result in complete dissolution of the matrix spots and must be avoided. The importance of using small sample volumes is illustrated in Figure 3. In both cases the same amount of sample is loaded on the matrix spot. However, the lower volume results in much better signal-to-noise ratios.

Sample Size Influences Signal Intensity

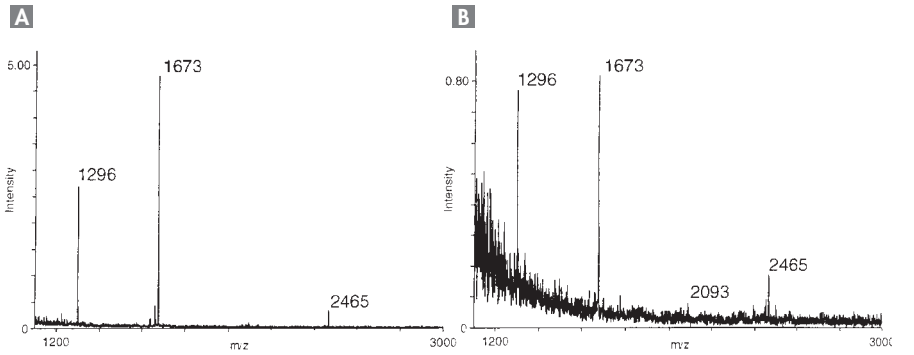


Figure 3 Lower sample volumes provide increased signal intensities. A total of 100 amol of a mixture of angiotensin I [m/z = 1296], neurotensin [m/z = 1673], and ACTH 18–39 [m/z = 2465] was spotted onto an Mass·Spec·Turbo Peptide Chip. **A** 0.1 µl of a 1 fmol/µl solution and **B** 1 µl of a 0.1 fmol/µl solution.

Certified materials and reagents

The list of certified materials and reagents in Table 1 contains items which have been demonstrated to be compatible with Mass·Spec·Turbo Chips. Use of these materials, or materials of a similar quality or purity, is necessary to obtain optimum performance.

Table 1. Materials and Reagents Certified for Use with Mass·Spec·Turbo Chips

Item	Recommended supplier
1.5 ml Safe-Lock Tubes	Eppendorf; cat no. 0030 120.086
Acetonitrile, HPLC grade	Applichem; cat. no. A3189,2500
Water, analytical grade	Merck; cat no. 1.16754.5000
Trifluoroacetic acid (TFA), analytical grade	Merck; cat no. 1.08262.0100
Pipet tips	BrandTech Scientific, cat. no. 702526 or Eppendorf AG, cat. no. 0030 073 380

Solvents

Because of the high MS sensitivity that is achieved by using Mass·Spec·Turbo Chips, it is critical that all solvents and reagents are of the highest purity. Lower-grade solvents interfere with MS performance. The solvents specified in Table 1 have been shown to enable crystallization and spectrum acquisition on Mass·Spec·Turbo Chips.

Plasticware

Many plastics used for tubes, pipet tips, etc., are incompatible with Mass·Spec·Turbo Chips because they may release plasticizers, polymers, or other stabilizing agents from the manufacturing process. This often results in the presence of strong polymer signals in MS spectra or poor to incomplete crystallization. For this reason, tubes (and other plasticware such as pipet tips) labelled as “low retention” or “siliconized” should be strictly avoided. We strongly recommend the use of the materials specified in Table 1 to avoid these potential problems.

Spot finishing on Mass·Spec·Turbo Chips

Before signal measurement, the spots are washed with a finishing solution. Spot finishing by re-crystallization using small liquid volumes is important to reduce the yield of matrix cluster ions and sodium and potassium adducts, and to obtain a reproducible and well-defined incorporation of the analyte within the matrix spots. In addition, this step significantly reduces matrix cluster peaks and increases signals from the analyte (see Figure 4).

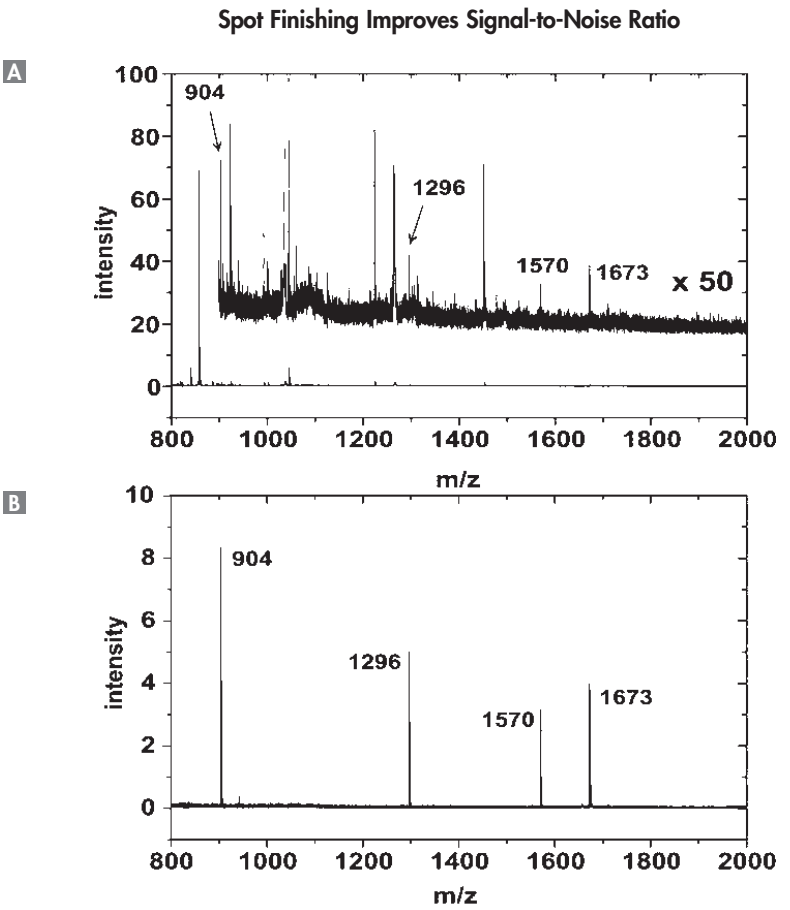


Figure 4 A Spectrum (insert x50) of a peptide mix (des Arg1 Bradykinin [m/z = 904]; Angiotensin 1 [m/z=1296]; Glu1-Fibrinopeptide B [m/z = 1570]; Neurotensin [m/z = 1673]) analyzed without performing the spot finishing step. B Spectrum of the same peptide mixture analyzed after performing the spot finishing step.

Design of Mass·Spec·Turbo Chips

Mass·Spec·Turbo Chips are disposable devices comprising matrix spots predeposited by vacuum sublimation onto an ultrahydrophobic surface. Compared to stainless steel MALDI targets this surface has reduced light-reflecting properties (see Troubleshooting Guide, page 26). It may therefore be difficult to see Mass·Spec·Turbo Chip matrix spots by video monitoring of the MALDI target. However, due to the fact that the prespotted matrix is very homogeneous and remains in situ during sample preparation, analysis of Mass·Spec·Turbo Chips does not require so-called "sweet-spot searching". Instead, it is highly recommended that measurements are performed in the automatic mode (see MALDI Analysis using Mass·Spec·Turbo Chips, page 20).

For automatic MALDI-MS measurements, chip alignment is performed using four alignment spots (AL1, AL2, AL3, and AL4). They are located outside the dark area of the spot array (see Figure 5). External mass calibration is performed with the six calibration spots (CAL1 – CAL6). These calibration spots come pre-spotted with a peptide calibration mix that contains the following peptides.

Peptide	m/z	Peptide	m/z
Des-Arg Bradykinin	904.47	ACTH (1–17)	2093.09
Angiotensin I	1296.69	ACTH (18–39)	2465.20
Glu[1]-Fibrinopeptide B	1570.68	ACTH (7–38)	3657.93
Neurotensin	1672.92		

On-Chip Alignment and Calibration Spots

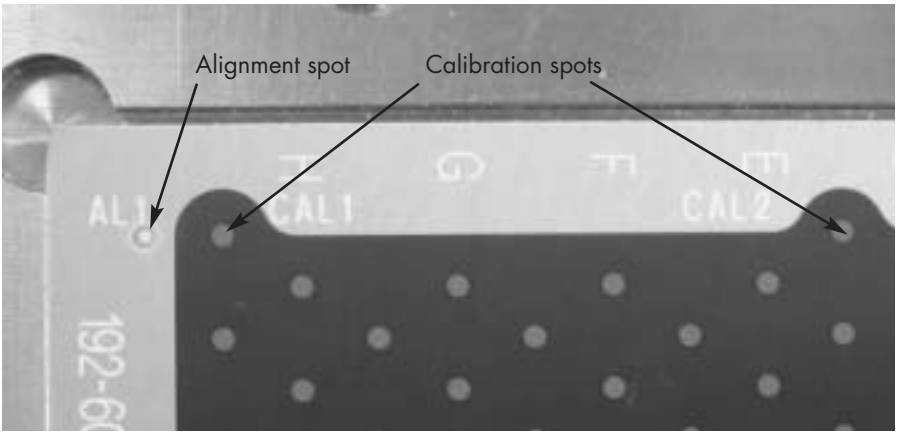


Figure 5 Alignment and calibration spots on the edge of Mass·Spec·Turbo Chips.

Protocol: Manual Spotting and Finishing of Mass·Spec·Turbo 192 Chips

This protocol is used for manual sample application and spot finishing on Mass·Spec·Turbo 192 Chips. To achieve optimum performance, follow all protocol steps and recommendations carefully and use only highest purity reagents (e.g., ultra-high purity [18 MΩ] water). Solution compositions can be found in Appendix A on page 29.

Important points before starting

- High proportions (≥50%) of acetonitrile (ACN) or high volumes of organic sample solutions can dissolve the pre-deposited spots completely. The dissolved matrix will re-crystallize as large crystals which are distributed irregularly over the previously covered area. This will destroy the advantages of the very homogenous crystallization reached by the sublimation of the matrix in the high-vacuum process. We strongly recommend following the recommended protocols to ensure that spots are not completely dissolved.
- MALDI signal intensities depend strongly on the sample volume applied and the proportion of ACN in the sample solvent. For high-sensitivity measurements, low volumes of highly concentrated samples should be applied using solvents containing low amounts of ACN. Typical working sample volumes are 0.1–2.0 µl for Mass·Spec·Turbo 192 chips. The maximum recommended volume is 10 µl for a solution containing 30% ACN/0.1% TFA.
- Sample solutions may contain low boiling point organic solvents and trifluoroacetic acid (TFA). These solutions should be used in well-ventilated areas. Wear the appropriate protective equipment during all procedures.
- Use plastic tweezers when handling chips and only grip the blue edges of the chips. Avoid touching the ultrahydrophobic chip surface with gloves or tweezers.
- Dust or foreign particles on the chip surface may interfere with the re-crystallization of the matrix after the sample is applied or may cause damage to the mass spectrometer. Remove any dust or foreign material before sample preparation or before MALDI measurements. Use a dust-shield to prevent any foreign material from contaminating the chip surface.
- If no internal standards are used, it is strongly recommended that the six calibrant spots (CAL1–6) are used for calibration standards.
- MALDI signal intensities depend strongly on the volume of solution left on the spot after the finishing step. For high-sensitivity measurements, volumes of finishing solution left on the spots should be as low as possible.

Procedure

1. **Prepare sample solutions containing 0–40% of ACN (v/v) and 100–60% of 0.1% TFA in pure water.**

   It is important to use the recommended certified reagents (see page 13). The proportion of ACN should not exceed 40% when using sample volumes of ≥1 µl per spot as spots may be completely dissolved. Dilute the sample solution with 0.1% TFA or use less volume (see Troubleshooting Guide, page 26).

2. **Apply 0.1–2.0 µl sample solution to the desired numbers of spots.**

   Use a dust-shield to minimize contamination by airborne particles. If possible, work under an extraction hood.

3. **Allow the samples to air-dry.**

   Use a dust-shield to minimize contamination by airborne particles. If possible, work under an extraction hood.

4. **Proceed with the spot finishing procedure, using either protocol step 5a or 5b.**

5a. **Using a pipet, finish spots individually by applying 1 µl of Finishing Solution to each spot. After 3 s, aspirate as much of the Finishing Solution as possible, and allow to air-dry.**

   This method is recommended for Mass·Spec·Turbo 192 Chips. Use only the Finishing Solution supplied with the chips.

5b. **Finish spots by dipping the whole Mass·Spec·Turbo chip (or the part of it to which samples have been applied) into Finishing Solution. After 3 s remove the chip from the Finishing Solution and clean and dry the blue edges of the chip with a clean tissue. Clean and dry the complete reverse side of the chip with a clean tissue, and allow the chip to air-dry.**

   This method is recommended for Mass·Spec·Turbo 625 and 1600 Chips, which have a spot diameter of 480 µm. It can also be used for Mass·Spec·Turbo 192 Chips with a spot diameter of 600 µm. Use only the Finishing Solution supplied with the chips.

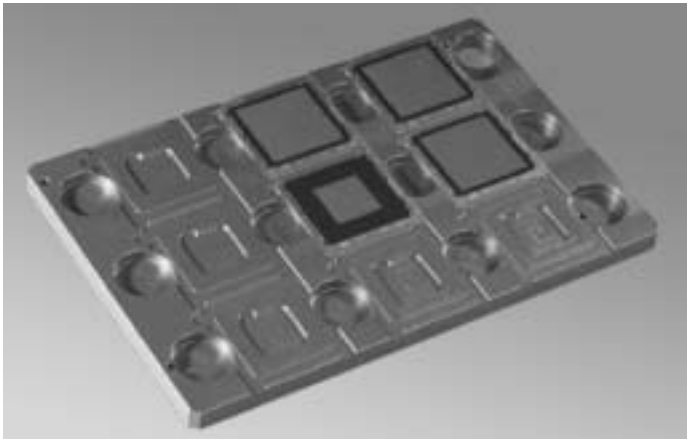
6. **Proceed with protocol "MALDI-MS Analysis Using Mass·Spec·Turbo Chips" on page 20.**

Protocol: Automated Preparation of Mass·Spec·Turbo Chips

Mass·Spec·Turbo Chips can be loaded using automated microfraction collectors or spotting robots. The protocol given here uses the Dionex Probot system as an example.

Procedure

1. **Mount the Mass·Spec·Turbo Chip in 1 of the 9 positions of the Probot Table (QIAGEN cat. no. 49902).**



Probot table

2. **Install the QIAGEN Sample Carrier Files* and QIAGEN Tray Files* on the computer used for controlling the Probot system.**

   Normally these files are located in *Program files/lcp/µcarrier/Sample Carriers or Trays*.

3. **Load the QIAGEN Tray File for the type of Mass·Spec·Turbo Chip being used.**
4. **Align the position of the chosen chip.**

   Be sure that the center of the capillary is in the middle of the upper-left spot. Check the alignment by moving to the lower-right spot after "homing" the system.

* These files are available to download at www.qiagen.com/maldiprep .

5. **Load user-developed applications both for the HPLC and the Probot systems.**

   Do not forget to switch off the syringe pump. Gradients should not exceed ACN levels of 60% when using sample volumes of 100 nl per spot. Spots may be completely dissolved.

6. **Start LC run.**

7. **Start Probot application.**

   Depending on your HPLC flow rate select the fraction collection time per spot in a range that 25–100 nl of sample solution are applied to each spot on Mass·Spec·Turbo 625 and 1600 Chips and 0.1–2.0 µl to each spot on Mass·Spec·Turbo 192 Chips.

8. **Let all samples air dry.**

9. **Proceed with the spot finishing procedure, using either protocol steps 10–11 or the automated protocol on page 19.**

   The automated procedure is strongly recommended, but is much more time consuming than the dipping method.

10. **Finish spots by dipping the whole Mass·Spec·Turbo chip (or the part of it to which samples have been applied) into Finishing Solution for 3 s.**

11. **After 3 s remove the chip from the Finishing Solution and clean and dry the blue edges of the chip with a clean tissue. Clean and dry the complete reverse side of the chip with a clean tissue, and allow the chip to air-dry.**

    This method is recommended for Mass·Spec·Turbo 625 and 1600 Chips. It can also be used for Mass·Spec·Turbo 192 Chips. Use only the Finishing Solution supplied with the chips.

Protocol: Automated Finishing of Mass·Spec·Turbo Chips

Spot finishing on Mass·Spec·Turbo Chips can be performed using automated microfraction collectors or spotting robots. The protocol given here uses the Probot system as an example.

Procedure

1. **Mount the Mass·Spec·Turbo Chip in 1 of the 9 positions of the Probot Table (QIAGEN cat. no. 49902).**
2. **Install the QIAGEN Sample Carrier Files* and QIAGEN Tray Files* on the computer used for controlling the Probot system.**

   Normally these files are located in *Program files/lcp/µcarrier/Sample Carriers or Trays*.
3. **Load the QIAGEN Tray File for the type of Mass·Spec·Turbo Chip being used.**
4. **Align the position of the chosen chip.**

   Be sure that the center of the capillary is in the middle of the upper-left spot. Check the alignment by moving to the lower-right spot after "homing" the system.
5. **Write or load an appropriate application for the Probot system.**

   Use a syringe pump for applying the Finishing Solution to the Mass·Spec·Turbo Chip.
6. **Start Probot application.**

   Depending on the flowrate of your syringe pump, select a fraction collection time that delivers 100 nl of Finishing Solution to each spot on Mass·Spec·Turbo 625 and 1600 Chips and 200 nl Finishing Solution to each spot on Mass·Spec·Turbo 192 Chips.
7. **Let all samples air-dry.**
8. **Proceed with protocol "MALDI-MS Analysis Using Mass·Spec·Turbo Chips" on page 20.**

* These files are available to download at www.qiagen.com/maldiprep.

MALDI-MS Analysis Using Mass·Spec·Turbo Chips

MALDI analysis of prepared spots on Mass·Spec·Turbo Chips can be performed on the following instruments:

Applied Biosystems

- 4700 Proteomics Analyzer
- Voyager DE Pro + STR
- QSTAR

Mass·Spec·Turbo Chips are used in conjunction with the Mass·Spec·Turbo Chip Holder 1 or Applied Biosystems Opti-TOF™ Holders. It is important that the type of chip holder matches the type of Mass·Spec·Turbo Chip being analyzed (see table below).

Table 2. Holder Requirements for Mass·Spec·Turbo Chip Analysis

Mass·Spec·Turbo Chips	Applied Biosystems 4700 Proteomics Analyzer	Applied Biosystems QSTAR or Voyager
Type 1: cat. nos. 49000, 49002, 49004, 49006, 49100	Mass·Spec·Turbo Chip Holder 1 (cat. no. 49910)	Not compatible
Type 2: cat. nos. 49001, 49003, 49005, 49107, 49101	Not compatible	Opti-TOF Holder for QSTAR and Voyager System*

* Applied Biosystems cat. no 4347689.

Important points before starting

- In some instruments — especially the Applied Biosystems 4700 and Voyager systems — it may be difficult to see spots due to the rough surface of the Mass·Spec·Turbo Chip. To view spots during MALDI measurements, video window adjustments or an additional external light source (e.g., a fiber optic cable) are necessary.
- On Mass·Spec·Turbo Chips, MALDI measurements can be performed in a dynamic range over four orders of magnitude. Depending on the instrument settings, a breakdown of linearity is observed above 100 fmol of analyte.
- MALDI signal intensities are strongly dependent on the instrument settings. For high-sensitivity measurements, adjustments to settings (e.g., laser intensity, number of shots, etc.) may need to be made. Usually only slight adjustments are necessary to achieve highest sensitivity. It is recommended that Mass·Spec·Turbo Tuning Chips are used to set reference parameters for optimized data acquisition. Follow the instructions for MALDI measurements which are described below. If signal intensities are not in the specified magnitude or if resolution is too low please contact the technical support department of your instrument vendor or QIAGEN. See "Troubleshooting Guide", page 26.
- To measure calibrant spots, follow the instructions for MALDI measurements which are described below.
- To achieve optimum performance, follow all protocol steps and recommendations carefully.
- Use plastic tweezers when handling chips and only grip the blue edges of the chips. Avoid touching the ultrahydrophobic chip surface with gloves or tweezers. Wear the appropriate protective equipment during all procedures.
- Dust or foreign particles on the chip surface may interfere with the re-crystallization of the matrix after the sample is applied or may cause damage to the mass spectrometer. Remove any dust or foreign material before sample preparation or before MALDI measurements. Use a dust-shield to prevent any foreign material from contaminating the chip surface.

Protocol: Analyzing Mass·Spec·Turbo Chips on the Applied Biosystems 4700 Proteomics Analyzer

For automatic MALDI-MS measurements, chip alignment is performed using four alignment spots (AL1, AL2, AL3, and AL4). They are located outside the dark area of the spot array. Usually, the alignment procedure must only be performed when changing chip formats.

Equipment to be supplied by user

- Chip holder: Mass·Spec·Turbo Chip Holder 1 (cat. no 49910)

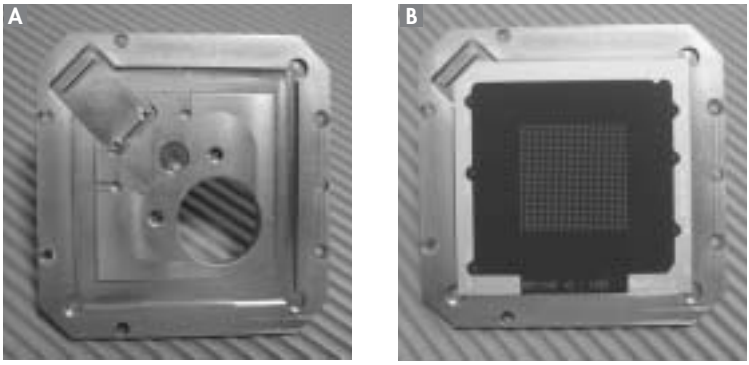


Figure 6 A Mass·Spec·Turbo Chip Holder 1 B with chip mounted.

Procedure

1. Insert Mass·Spec·Turbo Chip into the Chip Holder (see Figure 6).

2a. If using version 2.0 operating software, install QIAGEN Plate Files* on your computer and follow the steps below.

- Open Windows Explorer.
- Copy Plate Files.
- In Windows Explorer go to: C:\Program Files\Applied Biosystems\T-Squared\Unsupported Plate Files.
- Paste Plate Files.
- Open 4700 operating software.
- Open "Tools" in the task list.
- Got to "Load PLT-Files".
- Search for the required Plate File under the path mentioned above (C:\Program Files\...).
- Import the required Plate File.

* These files are available to download at www.qiagen.com/maldiprep .

2b. If using version 3.0 operating software install QIAGEN Plate Files* on your computer and follow the steps below.

- Open "File" in the task list.
- Go to "XML Database Import".
- Import required Template File.
- Repeat for each Template File.

3. Place the Chip Holder into the adapter and lower the laser intensity to 20% of the value normally used for sample measurements.

4. From the menu 'Plate' select 'Align Sample Plate'.

A pop-up menu opens. Follow the instructions.

5. Move the video window to find the alignment spot AL1.

The four alignment spots are located outside the ultrahydrophobic area. AL1 is located in the bottom left-hand corner or the chip. The areas around these spots are treated with a laser so that their surface is smooth and reflects light, making them easily visible. If the alignment spots cannot be seen, right-click in the video window and adjust brightness and contrast settings.

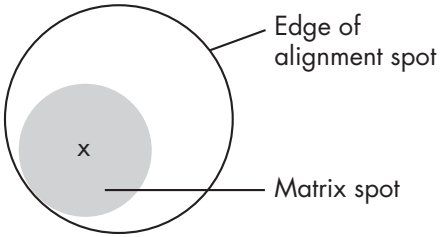
6. Center the focus of the laser on the center of the matrix spot which is located within the alignment spot (marked X below).

The matrix spot appears darker than the surrounding alignment spot. Do not focus the laser in the center of the alignment spot, because alignment will not be executed properly!

7. Repeat the alignment procedure for spots AL2 – AL4. Click 'Finish' when alignment procedure is completed.

8. Adjust laser intensity to 100% and perform MALDI measurements.

Note that less laser intensity and fewer laser shots are required to obtain the same spectrum quality as with standard stainless steel targets. Adjust other instrument settings where necessary.



* These files are available to download at www.qiagen.com/maldiprep .

Protocol: Analyzing Mass·Spec·Turbo Chips on the Applied Biosystems Voyager and QSTAR Instruments

Equipment to be supplied by user

- Chip holder: Opti-TOF Holder for QSTAR and Voyager System (Applied Biosystems cat. no 4347689)

Procedure

1. **Insert Mass·Spec·Turbo Chip into the chip holder (see Figure 7).**

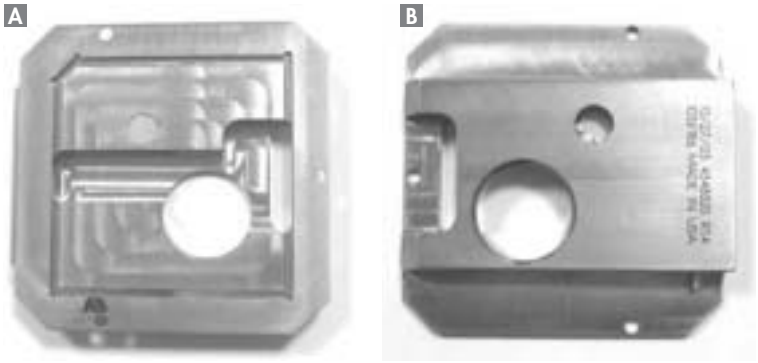


Figure 7 A Front and B reverse side of Opti-TOF Holder for QSTAR and Voyager System.

2. **Install QIAGEN Plate Files* on your computer and follow the steps below.**
   - Open Windows Explorer.
   - Copy Plate Files.
   - In Windows Explorer go to: C:\Program Files\Applied Biosystems\T-Squared\Unsupported Plate Files.
   - Paste Plate-Files.
   - Open 4700 operating software.
   - Open "Tools" in the task list.
   - Got to "Load PLT-Files".
   - Search for the needed Plate-File under the path mentioned above (C:\Program Files\...).
   - Import the Plate File.

* These files are available to download at www.qiagen.com/maldiprep .

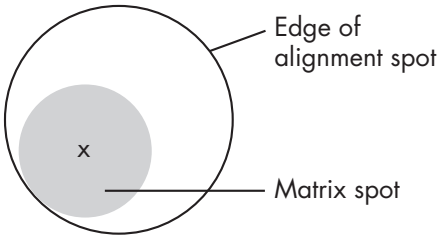
3. **Place the Chip Holder into the adapter and lower the laser intensity to 20% of the value normally used for sample measurements.**
4. **From the menu 'Plate' select 'Align Sample Plate'.**

   A pop-up menu opens. Follow the instructions.
5. **Move the video window to find the alignment spot AL1.**

   The four alignment spots are located outside the ultrahydrophobic area. AL1 is located in the bottom left-hand corner or the chip. The areas around these spots are treated with a laser so that their surface is smooth and reflects light, making them easily visible. If the alignment spots cannot be seen, right-click in the video window and adjust brightness and contrast settings.
6. **Center the focus of the laser on the center of the matrix spot which is located within the alignment spot (marked X below).**

   The matrix spot appears darker than the surrounding alignment spot. Do not focus the laser in the center of the alignment spot, because alignment will not be executed properly!
7. **Repeat the alignment procedure for spots AL2 – AL4. Click 'Finish' when alignment procedure is completed.**
8. **Adjust laser intensity to 100% and perform MALDI measurements.**

   Note that less laser intensity and fewer laser shots are required to obtain the same spectrum quality as with standard stainless steel targets. Adjust other instrument settings where necessary.



Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise during sample preparation, spot finishing step, and MALDI analysis. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information or protocols in this handbook or molecular biology applications (see back page for contact information).

		Comments and suggestions
Sample preparation		
a)	Spots dissolve completely	Organic content of sample solution is too high. Dilute sample solution with 0.1% TFA. Sample volume too high. Use lower sample volumes. Wrong solvent was used. Use only specified solvents. Even slight amounts of methanol will dissolve the spot completely. Ensure that LC-MALDI capillary is sufficiently purged after cleaning it with methanol.
b)	Prepared spots do not air-dry	Sample solution was contaminated with polymers. Use only specified plasticware. If the problem reoccurs, use new vials or tips for sample preparation. Wrong solvent used. Use only specified solvents. Solvents like DMSO have high boiling points and evaporate very slowly. The result will be poor crystallization.
c)	Spots are not contained by hydrophobic surface coating	Hydrophobic coating is damaged. Store the Mass·Spec·Turbo Chips under dark and dry conditions. Avoid exposure to sunlight. Do not damage the surface by touching with gloves or pipet tips.
d)	Problems encountered during spotting	Pipet tips are unsuitable. Use only specified plasticware.
e)	Spots not covered completely by sample solution	Sample volume is too low. Use larger sample volumes.

		Comments and suggestions
f)	Spots were missed during an LC run	Capillary was incorrectly adjusted. Align the Mass·Spec·Turbo Chip properly before the LC run. Sample solution wets the outside of the capillary; no drop is formed at the end of the capillary. Apply a hydrophobic coating (e.g., Repel-Silane ES, Amersham cat. no. 17-1332-01) to the outside of the capillary.
g)	Spots were damaged during an LC run	Capillary was incorrectly adjusted. Align the Mass·Spec·Turbo Chip properly before the LC run.
Spot finishing procedure		
a)	Prepared spots do not air-dry	Sample solution was contaminated with polymers. Use only specified plasticware. If the problem reoccurs, use new vials or tips for sample preparation. Wrong solvent used. Use only specified solvents. Solvents like DMSO have high boiling points and evaporate very slowly. The result will be poor crystallization.
b)	Spots are not contained by hydrophobic surface coating	Hydrophobic coating is damaged. Store the Mass·Spec·Turbo Chips under dark and dry conditions. Avoid exposure to sunlight. Do not damage the surface by touching with gloves or pipet tips.
c)	Problems encountered during spotting	Pipet tips are unsuitable. Use only specified plasticware.
d)	Spots not covered completely by Finishing Solution	Finishing Solution volume is too low. Use larger Finishing Solution volumes.
e)	Spots were not finished during an LC run	Capillary was incorrectly adjusted. Align the Mass·Spec·Turbo Chip properly before the LC run. Finishing Solution wets the outside of the capillary; no drop is formed at the end of the capillary. Apply a hydrophobic coating (e.g., Repel-Silane ES, Amersham cat. no. 17-1332-01) to the outside of the capillary.
f)	Spots were damaged during an LC run	Capillary was incorrectly adjusted. Align the Mass·Spec·Turbo Chip properly before the LC run.
g)	Spots were covered with a layer of salt after finishing	Incorrect salt concentration in the solution. Use only the Finishing Solution supplied with the chips.

MALDI analysis

a)	Spots cannot be seen in the video window.	Illumination of the system is too weak. Adjust video settings for higher brightness and contrast by right-clicking within the video window. Install an external light source.
b)	Alignment spot is not visible	Alignment spot lies outside the view of the video window. Move the joystick of the MALDI instrument to locate spot. Installation of an external light source will facilitate the search.
c)	Most of the signals have the same high signal intensity	Signals are saturated. Lower sample concentration, laser intensity, or number of shots per spectrum.
d)	No signals detectable or low signal-to-noise ratios	Sample concentrations are below detection limit. Increase sample concentration, laser intensity, or number of shots per spectrum.
		Instrument settings are incorrect. It is recommended that Mass·Spec·Turbo Tuning Chips are used to set reference parameters for optimized data acquisition. If signal intensities are not of the specified magnitude or if resolution is too low please contact the technical support department of your instrument vendor or QIAGEN. See page 31 for more information and sample spectra.
		Salt content of sample solution was too high. A thick salt layer inhibits the acquisition of MALDI spectra. Repeat the spot finishing step.
e)	44 or 58 Da ladder in spectrum	Plasticware was contaminated. Use only specified plasticware.
		Ultrahydrophobic surface was touched with a glove. Do not touch ultrahydrophobic surface with gloves. Do not prepare or analyze touched spots.
f)	Peak adducts of +22 and/or +39 Da	The analyte or finishing solution was contaminated with salt. Repeat finishing step. Repeat preparation with new sample and/or Finishing Solution.
g)	High-intensity matrix adduct signals appear in spectrum in the 600–1200 Da range	Spot finishing step was not properly performed. Repeat finishing step.

Appendix A: Solvent Solution Compositions

Solvent solution	For 1 ml
0.1% TFA	999 µl water; 1 µl TFA
10% ACN/0.1% TFA	899 µl water; 1 µl TFA; 100 µl ACN
20% ACN/0.1% TFA	799 µl water; 1 µl TFA; 200 µl ACN
30% ACN/0.1% TFA	699 µl water; 1 µl TFA; 300 µl ACN

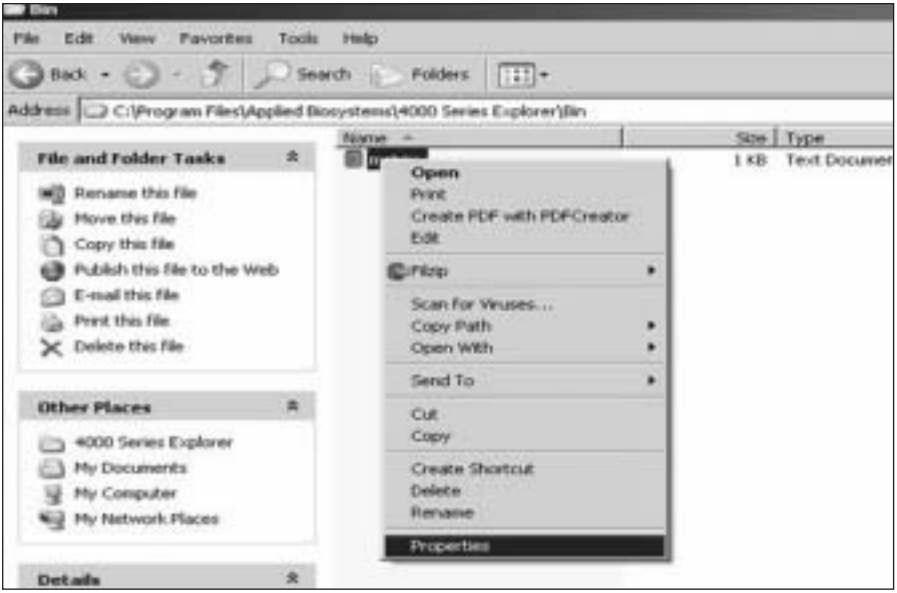
Appendix B: Changing the matrix.txt File in the Applied Biosystems 4000 Series Operating Software

For optimal results in MS/MS mode using Mass·Spec·Turbo Chips, the "Velocity" value that is read from the matrix.txt file in the instrument operating software must be set at 650. Follow the protocol beneath to set the velocity value.

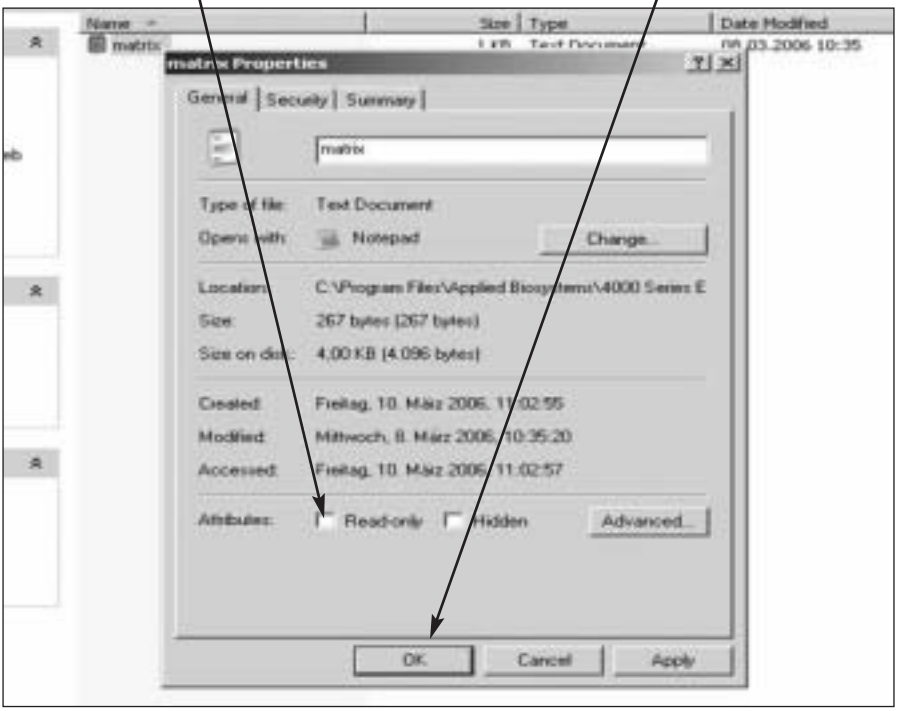
1. **On the computer used to operate the instrument, access the file C:\Program Files\Applied Biosystems\4000 Series Explorer\Bin in the "My Computer" directory.**

   Before making changes to the file, save a copy on the desktop.

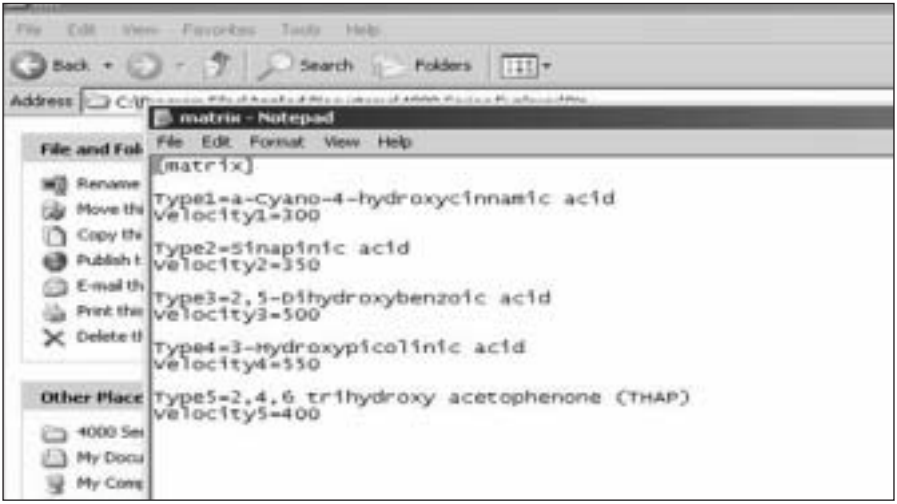
2. **Right-click on the file matrix.txt and select "Properties" from the drop-down menu.**



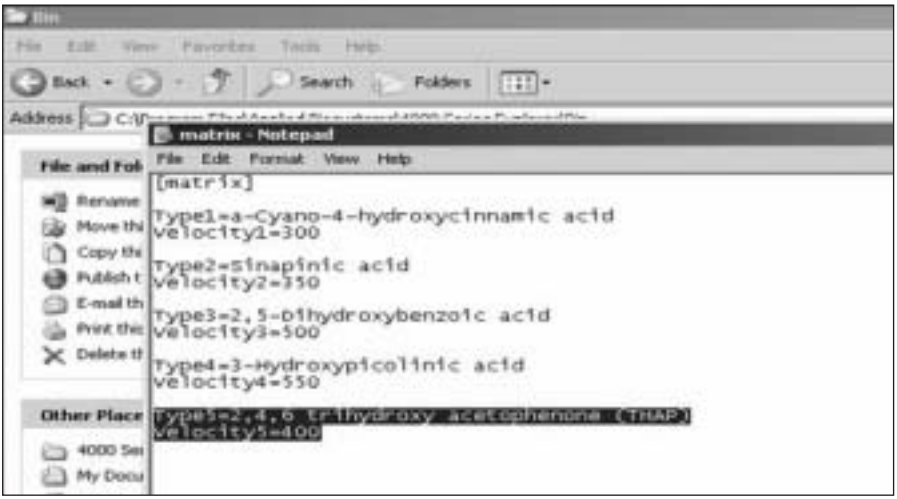
3. **Uncheck "Read-only" in the "Attributes:" field and click "OK".**



4. **Open the file matrix.txt with notepad by double-clicking on it in the file list, or by right-clicking and choosing Notepad from the "Open With" selection list.**

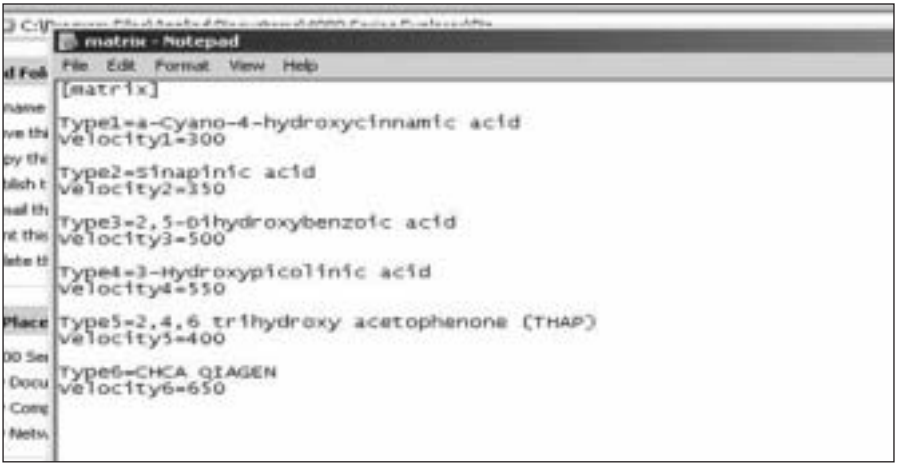


5. **Highlight the last two lines of text and copy them to the clipboard.**

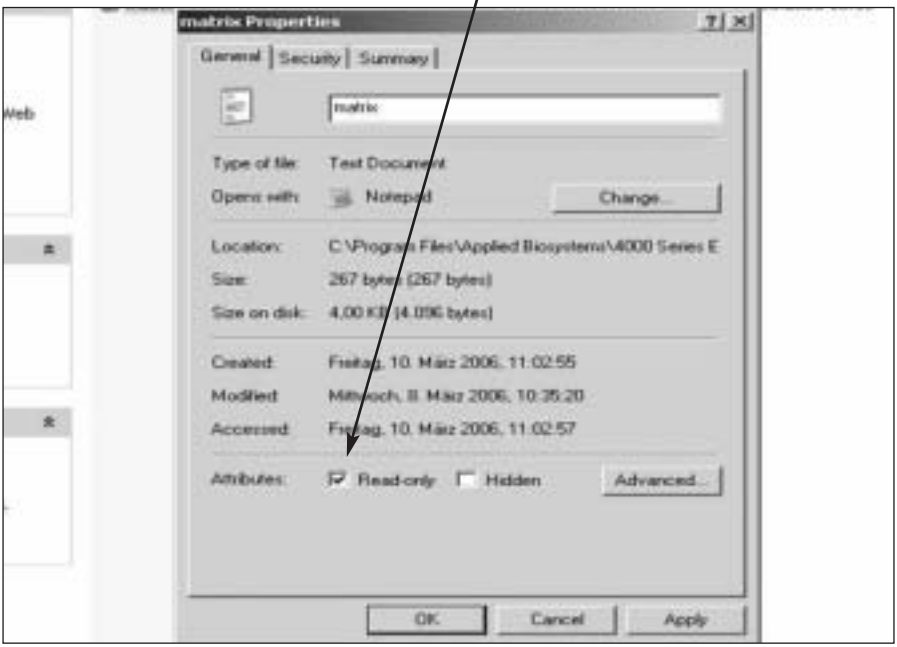


6. **Paste the two copied lines at the bottom of the document.**

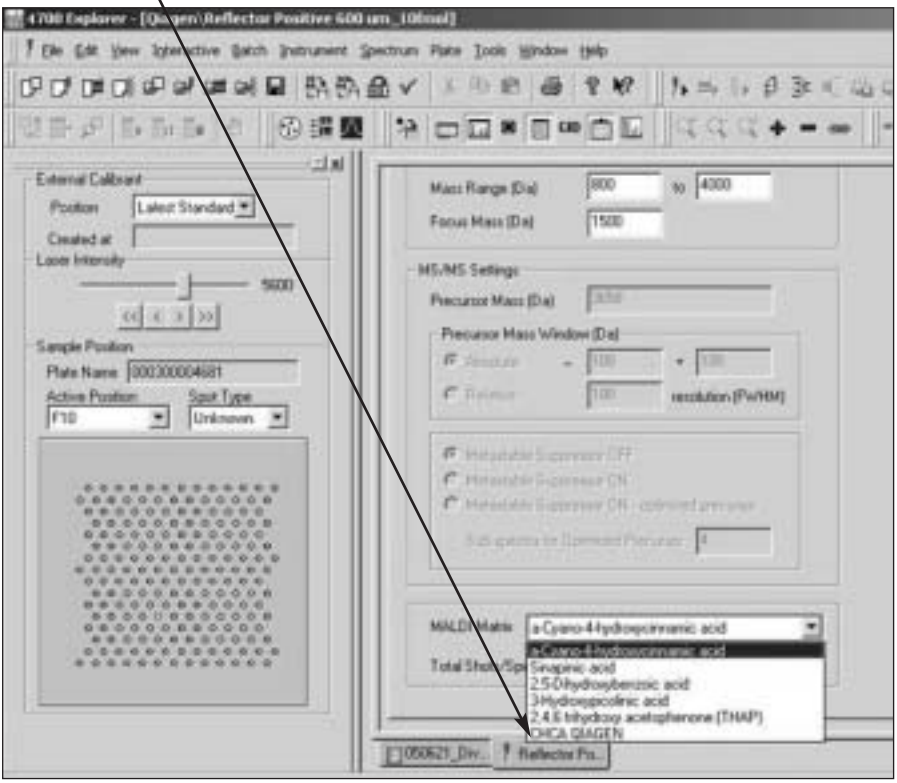
7. **Change the pasted text to read:**
   Type6=CHCA QIAGEN
   Velocity6=650



8. **Save the amended file, recheck the "Read-only" box in the "Properties" window, and click "OK".**



9. **Close the matrix.txt file and restart to 4000 Series Explorer software. The entry "CHCA QIAGEN" should now appear in the MALDI matrix drop-down menu.**



Ordering Information

Product	Contents	Cat. no.
Mass·Spec·Turbo 192 Peptide Chip 1	For use on Applied Biosystems 4700 MALDI-MS instruments: 2 chips each with 192 CHCA matrix spots and 6 calibration spots, Finishing Solution (2 x 100 ml)	49000
Mass·Spec·Turbo 192 Peptide Chip 2	For use on Applied Biosystems QSTAR and Voyager MALDI-MS instruments: 2 chips each with 192 CHCA matrix spots and 6 calibration spots, Finishing Solution (2 x 100 ml)	49001
Mass·Spec·Turbo 625 Peptide Chip 1	For use on Applied Biosystems 4700 MALDI-MS instruments: 2 chips each with 625 CHCA matrix spots and 6 calibration spots, Finishing Solution (2 x 100 ml)	49002
Mass·Spec·Turbo 625 Peptide Chip 2	For use on Applied Biosystems QSTAR and Voyager MALDI-MS instruments: 2 chips each with 625 CHCA matrix spots and 6 calibration spots, Finishing Solution (2 x 100 ml)	49003
Mass·Spec·Turbo 1600 Peptide Chip 1	For use on Applied Biosystems 4700 MALDI-MS instruments: 2 chips each with 1600 CHCA matrix spots and 6 calibration spots, Finishing Solution (2 x 100 ml)	49004
Mass·Spec·Turbo 1600 Peptide Chip 2	For use on Applied Biosystems QSTAR and Voyager MALDI-MS instruments: 2 chips each with 1600 CHCA matrix spots and 6 calibration spots, Finishing Solution (2 x 100 ml)	49005
Mass·Spec·Turbo 192 Protein Chip 1	For use on Applied Biosystems 4700 MALDI-MS instruments: 2 chips each with 192 sinapinic acid matrix spots and 6 calibration spots, Finishing Solution (2 x 100 ml)	49100
Mass·Spec·Turbo 192 Protein Chip 2	For use on Applied Biosystems QSTAR and Voyager MALDI-MS instruments: 2 chips each with 192 sinapinic acid matrix spots and 6 calibration spots, Finishing Solution (2 x 100 ml)	49101

Ordering Information

Product	Contents	Cat. no.
Mass·Spec·Turbo Chip Holder 1	Holder for Mass·Spec·Turbo Type 1 Chips: For use on Applied Biosystems 4700 MALDI-MS instruments	49910
Mass·Spec·Focus Chip 1	For Shimadzu Kratos MALDI-MS instruments: 6 chips with 16 wells for on-chip concentration of MALDI samples	49201
Mass·Spec·Focus Chip 2	For Waters MALDI-MS instruments: 1 chip with 96 wells for on-chip concentration of MALDI samples	49202
Mass·Spec·Focus Chip 3	For Applied Biosystems MALDI-MS instruments: 1 chip with 25 wells for on-chip concentration of MALDI samples	49203
Mass·Spec·Focus Chip 4	For Applied Biosystems MALDI-MS instruments: 1 chip with 64 wells for on-chip concentration of MALDI samples	49204
Mass·Spec·Focus Chip 5	For Thermo Electron MALDI-MS instruments: 6 chips with 16 wells for on-chip concentration of MALDI samples	49205
Mass·Spec·Focus Chip 6	For Bruker Daltonics MALDI-MS instruments: 6 chips with 16 wells for on-chip concentration of MALDI samples	49206
Mass·Spec·Focus Desalting Chip 1	For Shimadzu Kratos MALDI-MS instruments: 6 chips with 16 wells for on-chip cleanup and concentration of MALDI samples	49300
Mass·Spec·Focus Desalting Chip 2	For Waters MALDI-MS instruments: 1 chip with 96 wells for on-chip cleanup and concentration of MALDI samples	49301
Mass·Spec·Focus Desalting Chip 3	For Applied Biosystems MALDI-MS instruments: 1 chip with 25 wells for on-chip cleanup and concentration of MALDI samples	49302
Mass·Spec·Focus Desalting Chip 4	For Applied Biosystems MALDI-MS instruments: 1 chip with 64 wells for on-chip cleanup and concentration of MALDI samples	49303

Ordering Information

Product	Contents	Cat. no.
Mass·Spec·Focus Desalting Chip 5	For Thermo Electron MALDI-MS instruments: 6 chips with 16 wells for on-chip cleanup and concentration of MALDI samples	49304
Mass·Spec·Focus Desalting Chip 6	For Bruker Daltonics MALDI-MS instruments: 6 chips with 16 wells for on-chip cleanup and concentration of MALDI samples	49305
Mass·Spec·Focus IMAC Chip 1	For Shimadzu Kratos MALDI-MS instruments: 6 chips with 16 wells for on-chip IMAC purification and concentration of MALDI samples	49400
Mass·Spec·Focus IMAC Chip 2	For Waters MALDI-MS instruments: 1 chip with 96 wells for on-chip IMAC purification and concentration of MALDI samples	49401
Mass·Spec·Focus IMAC Chip 3	For Applied Biosystems MALDI-MS instruments: 1 chip with 25 wells for on-chip IMAC purification and concentration of MALDI samples	49402
Mass·Spec·Focus IMAC Chip 4	For Applied Biosystems MALDI-MS instruments: 1 chip with 64 wells for on-chip IMAC purification and concentration of MALDI samples	49403
Mass·Spec·Focus IMAC Chip 5	For Thermo Electron MALDI-MS instruments: 6 chips with 16 wells for on-chip IMAC purification and concentration of MALDI samples	49404
Mass·Spec·Focus IMAC Chip 5	For Thermo Electron MALDI-MS instruments: 6 chips with 16 wells for on-chip IMAC purification and concentration of MALDI samples	49405
Mass·Spec·Focus Chip Solvent Kit	For 1000 MALDI-MS measurements using Mass·Spec·Focus Chips: Ethanol, 0.1% TFA, acetonitrile, CHCA, DHB,ammonium citrate, peptide standard, tubes	49200

Notes

Notes

QIAGEN Companies

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Belgium ▪ Orders 0800-79612 ▪ Fax 0800-79611 ▪ Technical 0800-79556

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China ▪ Orders 021-51345678 ▪ Fax 021-51342500 ▪ Technical 021-51345678

Denmark ▪ Orders 80-885945 ▪ Fax 80-885944 ▪ Technical 80-885942

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Luxembourg ▪ Orders 8002-2076 ▪ Fax 8002-2073 ▪ Technical 8002-2067

The Netherlands ▪ Orders 0800-0229592 ▪ Fax 0800-0229593 ▪ Technical 0800-0229602

Norway ▪ Orders 800-18859 ▪ Fax 800-18817 ▪ Technical 800-18712

Sweden ▪ Orders 020-790282 ▪ Fax 020-790582 ▪ Technical 020-798328

Switzerland ▪ Orders 055-254-22-11 ▪ Fax 055-254-22-13 ▪ Technical 055-254-22-12

UK ▪ Orders 01293-422-911 ▪ Fax 01293-422-922 ▪ Technical 01293-422-999

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