

Third Edition

July 2006

Mass·Spec·Focus Chip Handbook

Mass·Spec·Focus Chips

Mass·Spec·Focus Desalting Chips

For preparation of MALDI mass
spectrometry samples



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Pack Contents

Mass-Spec-Focus Chips

| | Compatible instrument | Chips per pack | Sample wells per chip | Cat. no. |
|---------------|------------------------------|-----------------------|------------------------------|-----------------|
| Type 1 | Shimadzu Kratos | 6 | 16 | 49201 |
| Type 2 | Waters Corporation | 1 | 96 | 49202 |
| Type 3 | Applied Biosystems | 1 | 25 | 49203 |
| Type 4 | Applied Biosystems | 1 | 64 | 49204 |
| Type 5 | Thermo Electron | 6 | 16 | 49205 |
| Type 6 | Bruker Daltonics | 6 | 16 | 49206 |

Mass-Spec-Focus Desalting Chips

| | Compatible instrument | Chips per pack | Sample wells per chip | Cat. no. |
|---------------|------------------------------|-----------------------|------------------------------|-----------------|
| Type 1 | Shimadzu Kratos | 6 | 16 | 49300 |
| Type 2 | Waters Corporation | 1 | 96 | 49301 |
| Type 3 | Applied Biosystems | 1 | 25 | 49302 |
| Type 4 | Applied Biosystems | 1 | 64 | 49303 |
| Type 5 | Thermo Electron | 6 | 16 | 49304 |
| Type 6 | Bruker Daltonics | 6 | 16 | 49305 |

| Mass·Spec·Focus Chip Solvent Kit | Cat. no. 49200 |
|---|-----------------------|
| Acetonitrile | 30 ml |
| Ethanol | 10 ml |
| 0.1% TFA (trifluoroacetic acid) | 30 ml |
| CHCA (α -cyano-4-hydroxycinnamic acid) matrix | 100 mg |
| DHB (2,5-dihydroxybenzoic acid) matrix | 100 mg |
| Ammonium Citrate | 150 mg |
| 1.5 ml Microcentrifuge Tubes | 100 |
| Peptide Standard (ACTH 18–39, 10 nmols) | 1 vial |
| Handbook | 1 |

Storage and Stability

Mass·Spec·Focus Chips should be stored in their original packaging in dry conditions at room temperature (15–25°C) and protected from dust and light. After initial opening, the foil pouch should be kept closed and the chips stored under argon or nitrogen to prolong their shelf life.

Mass·Spec·Focus Chips can be stored under these conditions for up to 6 months without showing any decrease in performance.

Note: Unused wells on the chip surface are altered by the extreme vacuum conditions during MALDI analysis. Therefore, it is not advisable to repeatedly use part of a chip, perform analysis, remove the chip, and perform subsequent analyses in unused wells. Where possible, all wells of a chip should be used and analyzed in the same session. However if this is not possible, all wells on a chip should be used within a maximum of five sessions (Mass·Spec·Focus Chips) or three sessions (Mass·Spec·Focus Desalting Chips). In addition, the amount of time that chips spend in the instrument should be limited to that required for analysis.

Once a well has had matrix applied, the surface is no longer affected by the vacuum conditions in the instrument and the well can be archived and repeatedly analyzed. Archived chips should be stored at room temperature (15–25°C) in the dark in their original packaging, in dry conditions and protected from dust.

Reagents in the Mass·Spec·Focus Chip Solvent Kit should be stored at room temperature (15–25°C).

Quality Control

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

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.

The following risk and safety phrases apply to the Mass-Spec-Focus Solvent Kit components:

Ethanol: Contains ethanol. Highly flammable. Risk and safety phrases:* R11. S7-16.

Acetonitrile: Harmful. Highly flammable. Risk and safety phrases:* R11-20/21/22-36. S16-36/37.

Ammonium citrate: Irritant. Risk and safety phrases:* R36. S36.

α -cyano-4-hydroxycinnamic acid: Irritant. Risk and safety phrases:* R36/37/38. S13-26-36/37/39-46.

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

* R11 Highly flammable. R20/21/22 Harmful by inhalation, in contact with skin and if swallowed. R36 Irritating to eyes. R36/37/38 Irritating to eyes, respiratory system and skin. S7 Keep container tightly closed. S13 Keep away from food, drink and animal feedingstuffs. S16 Keep away from sources of ignition – No smoking. S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S36/37 Wear suitable protective clothing and gloves. S36/37/39 Wear suitable protective clothing, gloves and eye/face protection. S46 If swallowed, seek medical advice immediately and show container or label.

Product Use Limitations

Mass-Spec-Focus 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-Focus 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-Focus Chips are used for preparation and highly sensitive analysis of MALDI-MS samples. The ready-to-use targets provide on-chip concentration and cleanup of proteomics samples (e.g., tryptic digests from 2D gel spots) and offer several advantages over conventional stainless steel MALDI targets. These include efficient concentration of dilute sample solutions — which enables use of larger volumes and lowers limits of detection for easier identification of low-abundance peptides, and on-chip sample processing — which reduces handling, minimizes sample loss, and avoids compromising sample integrity.

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 and proteins. The main advantage of MALDI MS over conventional MS is that during the soft-ionization process, little or no fragmentation of analytes takes place, greatly facilitating identification of analyte molecular ions, even within mixtures. The technique has become a mainstay of proteomics research, due in large part to its ability to identify proteins from their characteristic peptide “fingerprints”.

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 formed from a dilute solution of the analyte in a matrix of molecules that efficiently absorb laser light. Such molecules include α -cyano-4-hydroxycinnamic acid (CHCA), sinapinic acid (SA), and 2,5-dihydroxybenzoic acid (DHB). 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. In the “plume” of matrix and analyte vapor generated above the target, analytes are protonated by the photo-excited matrix. As the ions are effectively generated instantaneously, they 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.

The sensitivity and speed of MALDI-MS analysis have led to its being used for the majority of protein and oligonucleotide analyses in high-throughput proteomics and genomics projects.

The Mass·Spec·Focus Chip principle

Mass·Spec·Focus chips are disposable devices for sample concentration and presentation for use with MALDI-MS. The chips contain a number of sites termed Chemically Defined Virtual Wells (CDVW, Figure 1).

Mass·Spec·Focus Chip Chemically Defined Virtual Wells

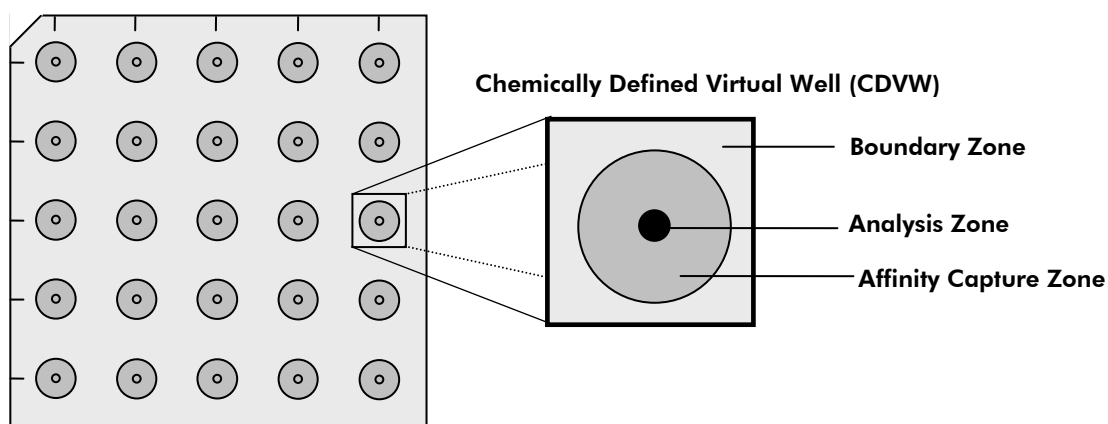


Figure 1 Structure of chemically defined virtual wells (shown here on a 5 x 5 Mass·Spec·Focus Type 3 Chip; see Appendix E for other formats).

Each well is created by the juxtaposition of proprietary surface chemistries on a flat surface in discrete concentric circular zones of wettability. The central, most wettable area is the Analysis Zone, which is surrounded by the less wettable Affinity Capture Zone. The Analysis Zone and Affinity Capture Zones are composed of differing chemistries that are designed to be resistant to analyte binding (Mass·Spec·Focus Chips) or to provide a surface for analyte binding (e.g., Mass·Spec·Focus Desalting Chips). Encircling both zones is the non-wettable Boundary Zone.

At first glance, it appears that Mass·Spec·Focus chips have no surface features. Depending on the type of chip (see Appendix E, page 34), registration marks on the edge of the chip may define the grid on which the centers of the wells are located. It is recommended that the site closest to a corner is used first, as they are the easiest to locate. Once this site contains liquid the positions of the other wells, which are based on the Society for Biomolecular Screening's standard 9 mm or 4.5 mm center-to-center spacing, are easy to locate.

Mass·Spec·Focus Chips — “on-chip” concentration of MALDI analytes

Using Mass·Spec·Focus chips, presentation and concentration of purified analytes is accomplished in a two-step process. After application, the analyte is concentrated into the Analysis Zone by the ‘focusing’ of liquid during solvent evaporation. After the sample dries, addition of matrix brings the analyte back into solution along with the matrix molecules. As the matrix solution dries, it focuses both matrix and analyte back into the Analysis Zone, where they co-crystallize. This process is illustrated in Figure 2.

Mass·Spec·Focus Chip Experimental Procedure

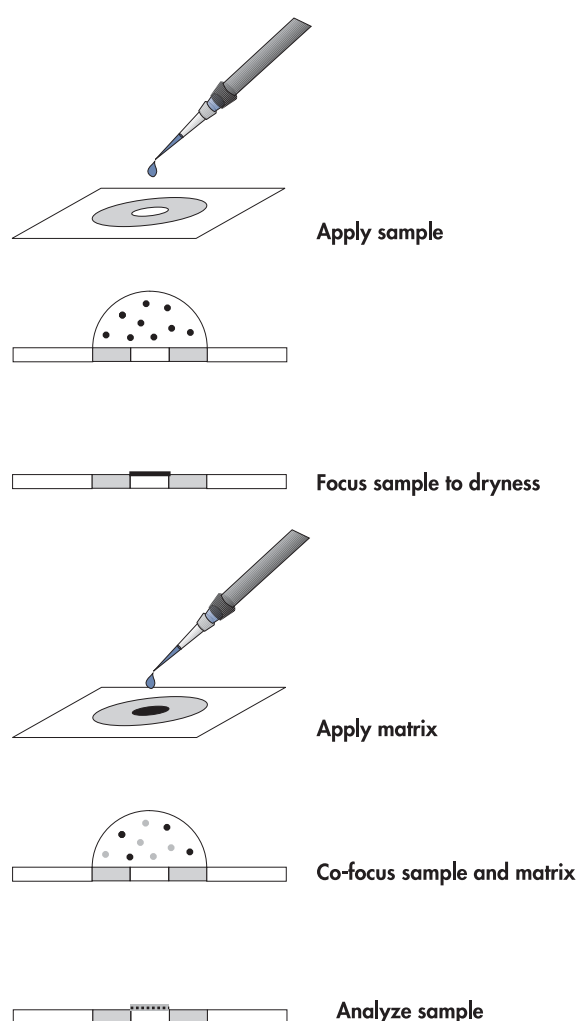


Figure 2 Workflow for sample analysis using Mass·Spec·Focus Chips.

Mass·Spec·Focus Desalting Chips — “on-chip” purification and concentration of MALDI analytes

Using the Mass·Spec·Focus Desalting Chip, purification and concentration of analytes is accomplished in a three-step process. Upon application, analytes are bound in the Affinity Capture Zone. Hydrophilic contaminants are then removed by washing the chip. Subsequent addition of matrix brings the analyte back into solution along with the matrix molecules. As the matrix solution dries, it crystallizes both matrix and analyte into the Analysis Zone. This process is illustrated in Figure 3.

Mass·Spec·Focus Desalting Chip Experimental Procedure

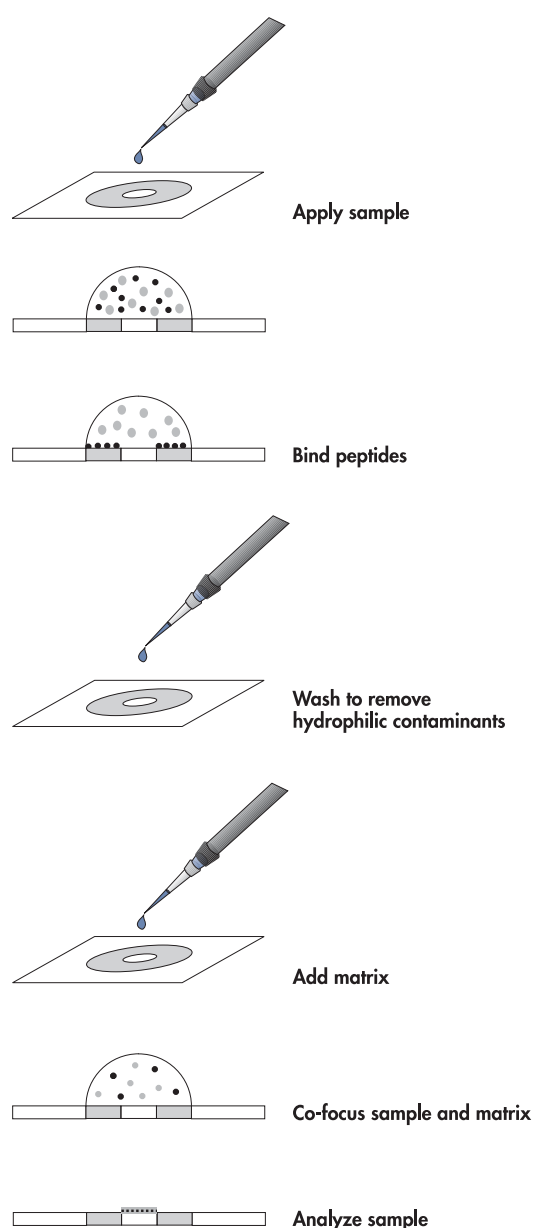


Figure 3 Workflow for sample analysis using Mass·Spec·Focus Desalting Chips.

Materials and reagents compatible with Mass·Spec·Focus Chips

The extreme sensitivity of MALDI-MS analysis requires that reagents used to prepare samples are of the highest quality. The reagents, tubes, and mixing vials in the Mass·Spec·Focus Chip Solvent Kit (cat. no. 49200) have been proven to deliver excellent results with Mass·Spec·Focus Chips. We strongly recommend using the materials in this kit to obtain optimal performance.

Solvents

Because of the concentrating effect of Mass·Spec·Focus Chips, it is critical that all solvents and reagents are of the highest purity. Lower-grade solvents interfere with both focusing and spectrum acquisition.

Plasticware

Many plastics are incompatible with Mass·Spec·Focus 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 focusing and crystallization. For this reason, tubes (and any other plasticware such as pipet tips) labeled as “Low-Retention” or “Siliconized” should be strictly avoided.

Peptide standard

The peptide ACTH (adrenocorticotrophic hormone) fragment 18–39 is provided in the Mass·Spec·Focus Solvent Kit for use as a reference standard. The fragment has the amino acid sequence Arg-Pro-Val-Lys-Val-Tyr-Pro-Asn-Gly-Ala-Glu-Asp-Glu-Ser-Ala-Glu-Ala-Phe-Pro-Leu-Glu-Phe and a calculated monoisotopic molecular weight of 2464.1989 Da.

The vial supplied in the Mass·Spec·Focus Solvent Kit contains 10 nmols of peptide. To prepare a stock solution of 100 pmol/ μ l dissolve the entire contents of the vial in 100 μ l 0.1% TFA. To prepare a stock solution of 10 pmol/ μ l dissolve the entire contents of the vial in 1000 μ l 0.1% TFA. Once reconstituted the stock solutions should be aliquoted and frozen. The capacity of chip wells is 1 pmol. To prepare a working solution, dilute stock solution to 0.1–1 pmol/ μ l and load an appropriate volume per well. For optimal results, do not subject peptide standard solutions to more than 3 freeze–thaw cycles.

Calibration wells

In addition to sample wells, Mass·Spec·Focus Chip types 1, 2, 5 and 6 contain calibration wells (see Appendix E, page 34). On type 1, 5, and 6 chips, the calibration wells contain only an Analysis Zone. Type 2 chip calibration wells have the same functionality (i.e., desalting or purification) as sample wells.

Reagents compatible with Mass-Spec-Focus Desalting Chips

Many substances used in proteomics or in the processing of MALDI samples can interfere with the MALDI-MS analysis procedure. For optimal results, samples must normally be cleaned up before analysis. The functionality of the Mass-Spec-Focus Desalting Chips allows sample purification and removal of these contaminants and detergents (up to a maximum concentration) on the chip. Some of the more commonly used reagents which can be effectively removed by Mass-Spec-Focus Desalting Chips chips are listed in the table below. Mass-Spec-Focus Desalting Chips do not allow removal of non-ionic reagents like Tween[®], Triton[®] X, or octyl-beta-glycoside (OBG).

Table 1. Reagents Removed by Mass-Spec-Focus Desalting Chips

| Reagent | Concentration |
|-----------------|----------------------|
| SDS | up to 0.1% |
| Urea | up to 1 M |
| Guanidinium-HCl | up to 4 M |
| NaCl | up to 1 M |
| Tris-HCl | up to 1 M |

Table 2. Materials and Reagents Certified for Use with QIAGEN Mass·Spec·Focus chips

| Item | Recommended supplier |
|--|--|
| Thermometer/Hygrometer | Fisher Scientific, cat no. 11.661.14 VWR, cat. no. 35519-049 Control Company, product number 4093 |
| 15 ml and 50 ml sterile (DNase and RNase free) PP-tubes | Greiner Bio-One GmbH, Frickenhausen, Germany; cat. no. 188 261 & 227 270 |
| LTS pipet tips (10, 250, and 1000 μ l) | Rainin Instrument, LLC., Woburn, MA, USA; cat. no. GPS-L10, GPS-L250, & GPS-L1000 |
| epTips pipet tips | Eppendorf AG, Hamburg, Germany or Brinkmann Instruments, Westbury, NY, USA |
| Safe-Lock microcentrifuge tubes (0.5 ml and 1.5 ml) | Eppendorf AG, Hamburg, Germany or Brinkmann Instruments, Westbury, NY, USA; cat. no. 22-36-361-1 & 22-36-320-4 |
| Ammonium acetate, SigmaUltra grade | Sigma-Aldrich, St. Louis, MO, USA; cat. no. A7330 |
| 3-(N-Morpholino)propane-sulfonic acid (MOPS), SigmaUltra grade | Sigma-Aldrich, St. Louis, MO, USA; cat. no. M5162 |

Measuring humidity

Crystallization of sample and matrix on Mass·Spec·Focus Chips is sensitive to humidity changes. Controlling humidity is important to the ultimate reproducibility of this process in the Analysis Zone. To check temperature and relative humidity (%RH) we strongly recommend using one of the combination thermometer/hygrometers from the Certified Materials list or an equivalent instrument. It will also help the user diagnose any problems outlined in the Troubleshooting Guide (see page 24).

Focusing matrix solutions in a humidity chamber

The process of matrix focusing is dependent on relative humidity; therefore the use of a hygrometer is highly recommended. Both CHCA and DHB will focus well if the relative humidity is between 50% and 70%. However, if the relative humidity is outside of this range then either a controlled humidity chamber (e.g., Mass·Spec·Focus Humidity Chamber, QIAGEN cat. no. 49903) and/or a refocusing solution may be needed. A simple controlled humidity chamber can be constructed by placing a water-saturated sponge in a box with a lid and walls at least 5 cm higher than the top of the sponge. Enough water should be added to the sponge to give a relative humidity of between 50% and 70%. The Chip is placed on top of the sponge for matrix application.

Protocol: Sample Application Using Mass·Spec·Focus Chips

Important points before starting

- Mass·Spec·Focus Chip wells have a capacity of approximately 1 pmol of analyte. If this amount is exceeded, the proper functioning of the chip will be compromised.
- The reagents, tubes, and mixing vials in the Mass·Spec·Focus Chip Solvent Kit (cat. no. 49200) have been proven to deliver excellent results with Mass·Spec·Focus Chips. We strongly recommend using the materials in this kit to obtain optimal performance.
- To achieve optimum performance, follow all protocol steps and recommendations carefully.
- Wear the appropriate personal protective equipment during all procedures.
- When handling Mass·Spec·Focus Chips use plastic tweezers and only grip the edges of the chip. Avoid touching the chip surfaces (especially the analysis and liquid retention zones) with gloves and tweezers.
- Dust or other foreign material on the chip surface — in particular on the liquid retention or analysis zones — may interfere with the focusing of either analyte or matrix solutions. The use of a dust shield is highly recommended to prevent any foreign material from contaminating the chip surface during processing.
- Typical working volumes for sample are 5–20 μl . Maximum recommended volumes for chips with 16 or fewer wells are:
 - 20 μl for a solution containing 50% acetonitrile; 0.1% TFA
 - 35 μl for 0.1% TFA
- It is strongly recommended that one or more sites on the chip are used for calibration with the peptide standard supplied in the Mass·Spec·Focus Solvent Kit (see page 13).
- Use only ultra-high purity (18 M Ω) water and the highest purity matrices (included in the Mass·Spec·Focus Chip Solvent Kit, cat. no. 49200) to ensure optimal performance.

- Unused wells on the chip surface are altered by the extreme vacuum conditions during MALDI analysis. Therefore, it is not advisable to repeatedly use part of a chip, perform analysis, remove the chip, and perform subsequent analyses in unused wells. Where possible, all wells of a chip should be used and analyzed in the same session. However if this is not possible, all wells on a Mass·Spec·Focus Chip should be used within a maximum of five sessions. In addition, the amount of time that chips spend in the instrument should be limited to that required for analysis. However, once sample and matrix are applied, the wells are no longer affected by the measurement conditions and chips can be archived and reanalyzed without problem.

Reagents and equipment to be supplied by user

- Mass·Spec·Focus Chip Solvent Kit, cat. no. 49200
- Optional: Thermometer/hygrometer and humidity chamber providing a relative humidity of 50–70%

Things to do before starting

- Using components of the Mass·Spec·Focus Chip Solvent Kit, (cat. no. 49200) prepare Sample Diluent — 25–50% (v/v) acetonitrile; 75–50% (v/v) 0.1% TFA. For example, mixing 4 volumes acetonitrile with 6 volumes 0.1% TFA gives a 40% (v/v) acetonitrile; 60% (v/v) 0.1% TFA solution.

Procedure

1. Prepare sample solutions containing 25–50% (v/v) acetonitrile and 50–75% (v/v) 0.1% TFA.

It is important to use the reagents from the Mass·Spec·Focus Solvent Kit (see 13).

2. Apply 1–20 μ l sample solution to each well.

Mass·Spec·Focus Chip wells have a capacity of approximately 1 pmol of analyte. If this amount is exceeded, the proper functioning of the chip will be compromised.

3. Focus analyte by allowing applied solutions to evaporate under ambient conditions or under reduced pressure.

Use a dust-shield to minimize contamination by airborne particles.

4. Proceed with the protocol “Co-Focusing Sample and Matrix on Mass·Spec·Focus Chips” on 20.

Protocol: Sample Application Using Mass·Spec·Focus Desalting Chips

There are three methods for applying analytes to Mass·Spec·Focus Desalting Chips.

- TFA “Wet” method — use this method for desalting analytes from solutions containing high concentrations of contaminants
- TFA “Dry Down” method — use this method for most standard applications and for desalting dilute analytes

An alternative MOPS method, which is used to supplement the peptide mass fingerprinting sequence coverage obtained with TFA methods, is provided in Appendix C on 28.

The sample and matrix co-focusing step is the same, regardless of the method used to deposit the analyte.

Important points before starting

- Mass·Spec·Focus Desalting Chip wells have a capacity of approximately 1 pmol of analyte. If this amount is exceeded, the proper functioning of the chip will be compromised.
- The reagents, tubes, and mixing vials in the Mass·Spec·Focus Chip Solvent Kit (cat. no. 49200) have been proven to deliver excellent results with Mass·Spec·Focus Chips. We strongly recommend using the materials in this kit to obtain optimal performance.
- To achieve optimum performance, follow all protocol steps and recommendations carefully.
- Wear the appropriate personal protective equipment during all procedures.
- When handling Mass·Spec·Focus Desalting Chips use plastic tweezers and only grip the edges of the chip. Avoid touching the chip surfaces (especially the analysis and liquid retention zones) with gloves and tweezers.
- Dust or other foreign material on the chip surface — in particular on the liquid retention or analysis zones — may interfere with the focusing of either analyte or matrix solutions. The use of a dust shield is highly recommended to prevent any foreign material from contaminating the chip surface during processing.
- Typical working volumes for sample are 5–20 μl . Maximum recommended volumes for chips with 16 or fewer wells are:
 - 20 μl for a solution containing 50% acetonitrile; 0.1% TFA
 - 35 μl for 0.1% TFA

- It is strongly recommended that one or more sites on the chip are used for calibration with the peptide standard supplied in the Mass·Spec·Focus Solvent Kit (see page 13).
- Use only ultra-high purity (18 MΩ) water and the highest purity matrices (included in the Mass·Spec·Focus Chip Solvent Kit, cat. no. 49200) to ensure optimal performance.
- Unused wells on the chip surface are altered by the extreme vacuum conditions during MALDI analysis. Therefore, it is not advisable to repeatedly use part of a chip, perform analysis, remove the chip, and perform subsequent analyses in unused wells. Where possible, all wells of a chip should be used and analyzed in the same session. However if this is not possible, all wells on a Mass·Spec·Focus Desalting Chip should be used within a maximum of three sessions. In addition, the amount of time that chips spend in the instrument should be limited to that required for analysis. However, once sample and matrix are applied, the wells are no longer affected by the measurement conditions and chips can be archived and reanalyzed without problem.

Reagents and equipment to be supplied by user

- Mass·Spec·Focus Chip Solvent Kit, cat. no. 49200
- Optional: Thermometer/hygrometer and humidity chamber providing a relative humidity of 50–70%

Things to do before starting

- Using components of the Mass·Spec·Focus Chip Solvent Kit, (cat. no. 49200) prepare Sample Diluent — 25–50% (v/v) acetonitrile; 75–50% (v/v) 0.1% TFA. For example, mixing 4 volumes acetonitrile with 6 volumes 0.1% TFA gives a 40% (v/v) acetonitrile; 60% (v/v) 0.1% TFA solution.

Procedure — TFA “Wet” Method

1a. Prepare sample solutions with Sample Diluent (25–50% [v/v] acetonitrile and 50–75% [v/v] 0.1% TFA).

It is important to use reagents from the Mass·Spec·Focus Solvent Kit (see page 13).

2a. Apply 5–20 μl sample solution to each well.

3a. Bind analyte by incubating applied solutions for 20 min.

Cover loosely to minimize contamination by airborne particles, but leave enough space to allow the acetonitrile to evaporate.

4a. Remove any residual solution with a pipet.

5a. Apply 10 μl of 0.1% TFA to each well.

- 6a. Immediately remove liquid with a pipet.**
- 7a. Repeat steps 5a – 6a twice.**
- 8a. Allow Mass·Spec·Focus Chip to air-dry.**
Use a dust-shield to minimize contamination by airborne particles.
- 9a. Proceed with the protocol “Co-Focusing Sample and Matrix on Mass·Spec·Focus Chips” on page 22.**

TFA “Dry Down” Method

- 1b. Prepare sample solutions with Sample Diluent (25–50% [v/v] acetonitrile and 50–75% [v/v] 0.1% TFA).**
It is important to use reagents from the Mass·Spec·Focus Solvent Kit (see page 13).
- 2b. Apply 5–20 μ l sample solution to each well.**
- 3b. Bind analyte by allowing applied solutions to dry down under ambient conditions.**
Use a dust-shield to minimize contamination by airborne particles.
- 4b. Apply 10 μ l of 0.1% TFA to each well.**
- 5b. Allow to incubate for 2 min and remove liquid with a pipet.**
- 6b. Repeat steps 4b – 5b twice.**
- 7b. Allow Mass·Spec·Focus Chip to air-dry.**
Use a dust-shield to minimize contamination by airborne particles.
- 8b. Proceed with the protocol “Co-Focusing Sample and Matrix on Mass·Spec·Focus Chips” on page 22.**

Protocol: Co-Focusing Sample and Matrix on Mass·Spec·Focus Chips

Use this protocol to co-focus analytes and matrix after analyte deposition. Either 2,5-Dihydroxybenzoic acid (DHB) or α -Cyano-4-hydroxycinnamic acid (CHCA) matrices (or both) can be used with Mass·Spec·Focus chips. The choice of matrix should be determined by the nature of the analysis, the analyte(s), and the user's instrument. CHCA and DHB and the solvents required to make a working solution are supplied in the Mass·Spec·Focus Chip Solvent Kit, cat. no. 49200.

Important points before starting

- The reagents, tubes, and mixing vials in the Mass·Spec·Focus Chip Solvent Kit (cat. no. 49200) have been proven to deliver excellent results with Mass·Spec·Focus Chips. We strongly recommend using the materials in this kit to obtain optimal performance.
- Matrix refocusing is dependent on relative humidity, and therefore use of a hygrometer is highly recommended. Both CHCA and DHB will focus well if the relative humidity is between 50% and 70%. However, if the humidity is outside this range, either a controlled humidity chamber (see page 16) and/or a refocusing solution may be needed.
- To achieve optimum performance, follow all protocol steps and recommendations carefully.
- Wear the appropriate personal protective equipment during all procedures.
- Matrix solutions contain low boiling point organic solvents and TFA. These solutions should be used in well ventilated areas.
- When handling Mass·Spec·Focus Chips use plastic tweezers and only grip the edges of the chip. Avoid touching the chip surface (especially the analysis and liquid retention zones) with gloves and tweezers.
- Dust or other foreign material on the chip surface — in particular on the liquid retention or analysis zones — may interfere with the focusing of either analyte or matrix solutions. The use of a dust shield is highly recommended to prevent any foreign material from contaminating the chip surface during processing.
- It is strongly recommended that one or more sites on the chip be used for calibration standards.
- Use only ultra-high purity (18 M Ω) water and the highest purity matrices (included in the Mass·Spec·Focus Chip Solvent Kit, cat. no. 49200) to ensure optimal performance.

Reagents and equipment to be supplied by user

- Matrix working solution, see Appendix D, page 32. All components are supplied in the Mass·Spec·Focus Chip Solvent Kit (cat. no. 49200).
- Optional: Thermometer/hygrometer and humidity chamber providing a relative humidity of 50–70%

Procedure

Co-focusing analytes and matrix

1. Apply 2 μ l of matrix working solution to each well containing analyte.

To avoid polymer contamination, rinse the pipet tip by pipetting matrix solution up and down several times before applying matrix solution to the chip. If necessary, use the pipet tip to gently move the matrix droplet around the well to fully wet the liquid retention zone.

2. Allow the matrix solution to focus and dry under ambient conditions and collect data.

If the matrix focuses properly, an almost perfectly circular matrix layer of 500–600 μ m diameter is formed in the central Analysis Zone. If the matrix spot diameter is much broader and/or the spot shows a pronounced frayed rim, the focusing has not worked properly, probably due to unsuitable ambient humidity levels. To refocus the matrix solution, proceed using steps 3a – 4a or 3b – 4b. For future analyses, consider using a humidity chamber (see page 16) to avoid refocusing.

3a. If the ambient relative humidity is less than 50%, place the chip in a humidity chamber (see page 16) and apply 1 μ l of 90:10 ACN:0.1% TFA refocusing solution to each well containing analyte.

To avoid polymer contamination, rinse the pipet tip by pipetting solution up and down several times before applying refocusing solution to the chip. Note that the refocusing solution does not contain ammonium citrate or matrix.

4a. Allow the matrix solution to refocus and dry in the humidity chamber and collect data.

OR

3b. If the ambient relative humidity is greater than 70%, apply 1 μ l of 98:2 ACN:0.1% TFA refocusing solution to each well containing analyte.

To avoid polymer contamination, rinse the pipet tip by pipetting solution in the reservoir up and down several times before applying refocusing solution to the chip. Note that the refocusing solution does not contain ammonium citrate or matrix.

4b. Allow the matrix solution to refocus at ambient conditions and collect data.

Troubleshooting Guide

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

Comments and suggestions

Contamination of spectrum by a polymer (i.e., a 44 or 58 Da ladder)

- | | |
|--|---|
| a) Plasticware was contaminated | Use recommended tubes (see page 15) and/or change pipet tip vendor. To avoid polymer contamination, rinse the pipet tip by pipetting solution up and down several times before applying solution to the chip. |
| b) Glove touched chip surface | Do not touch the surface of Mass·Spec·Focus Chips with gloves. |
| c) Sample is contaminated with a non-ionic detergent | Remove detergent from sample or prepare sample without using non-ionic detergent |

+22 and/or +39 Da peaks

- | | |
|--------------------|--|
| Salt contamination | Analyze sample using Mass·Spec·Focus Desalting Chip. |
|--------------------|--|

Matrix does not focus (see also page 26)

- | | |
|--|---|
| a) Excessive amount of analyte present | Mass·Spec·Focus Chip wells have a binding capacity of approximately 1 pmol of analyte. If this amount is exceeded, the proper functioning of the chip will be compromised. Repeat analysis using diluted analyte. |
| b) Dust contamination on Chip surface | Repeat analysis using a new chip and a dust-shield. |
| c) Wrong matrix solvent was used | Repeat analysis using the correct matrix solvent. |

Comments and suggestions

- d) Extreme humidity conditions in laboratory
- Measure relative humidity with a hygrometer.
- In the case of low relative humidity (<50%) use a humidity chamber and refocus matrix using 1 μ l 90:10 (ACN:0.1% TFA).
- In the case of high relative humidity (>70%) refocus matrix using 1 μ l 98:2 (ACN:0.1% TFA).

Poor matrix crystallization

- a) Polymer contamination
- Use only specified plasticware (see page 15). Do not use plasticware that has been sterilized, siliconized, or molded using releasing agents. To avoid polymer contamination, rinse the pipet tip by pipetting solution up and down several times before applying solution to the chip.
- b) Wrong matrix solvent was used
- Repeat analysis using the correct matrix solvent.
- c) Extreme humidity conditions in laboratory
- Measure relative humidity with a hygrometer.
- In the case of low relative humidity (<50%) use a humidity chamber and refocus matrix using 1 μ l 90:10 (ACN:0.1% TFA).
- In the case of high relative humidity (>70%) refocus matrix using 1 μ l 98:2 (ACN:0.1% TFA).

Poor performance with gel extracts

- Incompatibility of gel stain, digestion, or extraction procedures with Mass·Spec·Focus Chip
- Repeat using a Mass·Spec·Focus Desalting Chip.

Appendix A: Troubleshooting Decision Tree

Troubleshooting outline for poor focus and/or crystallization:

- 1. Is the relative humidity within the 50–70% range?**

Yes – Go to step 3.
No – Try the recommended refocusing step.
- 2. Does refocusing step work?**

Yes – Problem solved. Proceed with experiment, using refocusing step or humidity chamber.
No – Go to step 3 (using controlled % relative humidity for all subsequent steps if possible).
- 3. Does matrix solution focus and crystallize on chip without sample?**

Yes – Go to step 7.
No – Test matrix solvent solution on chip to see if it will focus. Go to step 4.
- 4. Does the matrix solvent solution focus on chip?**

Yes – Make fresh matrix solution, carefully following directions. Return to step 3.
No – Re-make matrix solvent solution, adding components in the specified order. Go to step 5.
- 5. Does the remade matrix solvent solution focus on chip?**

Yes – Make fresh matrix solution with remade solvent solution. Return to step 3.
No – Go to step 6.
- 6. Do the individual solvents ACN, EtOH, 0.1% TFA (without ammonium citrate!) focus on the chip, leaving no visible residue in the liquid retention zone?**

Yes – Re-make matrix solvent solution, carefully adding components in the specified order. Return to step 5.
No – Failing solvent is contaminated. Replace with higher purity material (if using recommended materials, take a fresh aliquot into recommended container). Repeat step 6.
- 7. Does sample solution focus on chip?**

Yes – Go to step 10.
No – Sample solution is contaminated, or is too concentrated (the capacity of the chip well is approximately 1 pmol of analyte). Dilute sample, go to step 8.

8. Does diluted sample solution focus on chip?

Yes – Go to step 11.

No – Test sample diluent. Go to step 9.

9. Does sample diluent focus on chip?

Yes – Sample is contaminated. Use a Mass·Spec·Focus Desalting Chip to clean up and focus sample.

No – Remake sample diluent with fresh aliquots of highest grade ACN and TFA, then make a fresh sample in the remade diluent. Return to step 8 (or step 7, if contaminated sample diluent was used to prepare initial sample).

10. Does matrix focus and crystallize on chip after sample has been applied and focused?

Yes – Problem solved. Proceed with the experiment.

No – Sample is either too concentrated or contaminated. Dilute sample, and go to step 11.

11. Does matrix focus and crystallize on chip after diluted sample has been applied?

Yes – Problem solved. Proceed with the experiment using diluted sample.

No – Sample is contaminated. Use Mass·Spec·Focus Desalting Chip to clean up and focus sample.

Appendix B: In-Gel Tryptic Digestion

This protocol can be used to prepare tryptic digests of proteins in gel spots or bands. The digests are analyzed using Mass·Spec·Focus Desalting Chips.

Mass·Spec·Focus Chip wells have a capacity of approximately 1 picomole of analyte. This corresponds to 50 ng of a 50 kDa protein. If this amount is exceeded, the proper functioning of the chip will be compromised. Please bear this in mind when processing gel slices where the total amount of protein in the band or spot should not exceed 1 picomole.

Following electrophoresis, the gel is stained using GelCode[®] Blue Stain Reagent from Pierce Biotechnology, Inc. (Cat. no. 24590).

Equipment and reagents to be supplied by user*

- GelCode Blue Stain Reagent
- Ultrapure water
- Shaker or rocker table
- Staining/destaining bath
- Reducing solution – 100 mM NH_4HCO_3 , 10 mM DTT
- Alkylation solution – 100 mM NH_4HCO_3 , 55 mM iodoacetamide
- 100 mM NH_4HCO_3
- 50 mM NH_4HCO_3
- Acetonitrile (100%)
- Destain solution – 50% acetonitrile in 100 mM NH_4HCO_3
- Trypsin (15 ng/ μl in 50 mM NH_4HCO_3)

Procedure

Staining and destaining

1. **Separate proteins by electrophoresis and remove gel from electrophoresis assembly.**
2. **Place gel into a container suitable for gel-staining. Add water until the gel is completely covered. Rinse gel on a rocker or shaker platform for 15 min, changing the water every 5 min.**
3. **Stain gel with GelCode Blue for 1 h.**

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

4. Destain gel background for 1 hour using several changes of water (Water Wash Enhancement™ step).
5. Excise protein bands/spots and cut each one into small pieces (approx. 1 mm x 1mm) using a sterile scalpel. Place the gel pieces from each band/spot into separate microcentrifuge tubes.
6. Add 200 μ l of acetonitrile to each tube, close the tube, and incubate at room temperature for 15 min. After incubation, remove liquid using a pipet and allow gel pieces to air-dry or dry under vacuum.

Because protein alkylation is performed during 2D-PAGE analysis, steps 7–12 can be omitted if spots from 2D gels are being processed.

Protein reduction

7. Add 200 μ l of 100 mM NH_4HCO_3 , 10 mM DTT to each tube and incubate for 30 min at 56°C. After incubation remove any excess solution using a pipet.
8. Repeat step 6 to dry the gel pieces again.

Protein alkylation

9. Add 200 μ l of 100 mM NH_4HCO_3 , 55 mM iodoacetamide to each tube and incubate for 20 min at room temperature (15–25°C) in the dark. After incubation remove any excess solution using a pipet.
10. Wash gel pieces with 150 μ l of 100 mM NH_4HCO_3 for 15 min at room temperature (15–25°C). After incubation remove any excess solution using a pipet.
11. Repeat step 6 to dry the gel pieces again.
12. If gel pieces still contain stain, add 500 μ l 50% acetonitrile in 50mM NH_4HCO_3 and incubate for 30 min at 37°C. After incubation remove any excess solution using a pipet. Repeat if necessary. Once gel pieces are completely destained, dry them with acetonitrile as in step 6.

Trypsin digestion

13. To each tube, add sufficient trypsin (15 ng/ μ l) in 50 mM NH_4HCO_3 to cover the gel pieces. Incubate at 4°C for 1 hour and remove any excess enzyme solution.

14. Add sufficient 50 mM NH_4HCO_3 to each tube to cover the gel pieces and incubate overnight at 37°C.

If the entire sample is to be loaded onto a single well, it is important that the volume of solution is kept as small as possible.

15. Remove the solution to a clean microcentrifuge tube.

This is the digested protein sample that will be applied to the Mass·Spec·Focus Desalting Chip.

16. Add acetonitrile to 25% of the total volume.

For example, if the digested protein sample has a volume of 15 μ l, add 5 μ l acetonitrile.

Appendix C: MOPS-Based Method for Sample Concentration and Cleanup Using Mass·Spec·Focus Desalting Chips

This method is used to supplement the peptide mass fingerprinting sequence coverage obtained with the TFA methods on pages 19–21.

Things to do before starting

- Prepare Sample Diluent — 25–50% (v/v) acetonitrile; 75–50% (v/v) 50 mM MOPS, pH 6.5. For example, mixing 4 volumes acetonitrile with 6 volumes 50 mM MOPS, pH 6.5 gives a 40% (v/v) acetonitrile; 60% (v/v) 0.1% 50 mM MOPS solution.

MOPS Method

- 1. Prepare sample solutions containing approximately 25–50% acetonitrile and 50–75% 50 mM MOPS, pH 6.5.**
It is important to use the recommended certified reagents (see page 15).
- 2. Apply 5–20 μ l sample solution to each well.**
- 3. Bind analyte by incubating applied solutions for 20 min.**
Cover loosely to minimize contamination by airborne particles.
- 4. Remove sample solution with a pipet.**
- 5. Immediately apply the same volume of 20 mM ammonium acetate.**
- 6. Immediately remove ammonium acetate with a pipet.**
- 7. Repeat steps 5–6.**
- 8. Allow chip to air-dry.**
Use a dust-shield to minimize contamination by airborne particles.
- 9. Proceed with the protocol “Co-Focusing Sample and Matrix on Mass·Spec·Focus Chips” on page 22.**

Appendix D: Reagent Compositions

For best results, reagents should be used on the day they are prepared.

DHB Matrix Working Solution (1.0 mg/ml in 84:13:3 acetonitrile : ethanol : 5 mM ammonium citrate in 0.1% TFA)

1. Prepare a 50 mM (= 11.3 mg/ml) ammonium citrate (dibasic) solution in 0.1 % TFA in a 1.5 ml microcentrifuge tube. Mix by vortexing.
2. Dilute the solution prepared in step 1 by a factor of ten. Pipet 100 μ l of 50 mM ammonium citrate into 900 μ l 0.1% TFA and mix by vortexing.
3. Make a solvent stock solution of 84% (v/v) acetonitrile: 13% (v/v) ethanol: 3% (v/v) 5 mM ammonium citrate in 0.1% TFA. Pipet 1000 μ l acetonitrile, 155 μ l ethanol, and 36 μ l 5 mM ammonium citrate in 0.1% TFA (prepared in step 2) into a 1.5 ml microcentrifuge tube and mix by vortexing.
4. Weigh out 3–8 mg DHB into an empty Matrix Mixing Vial and dissolve in the amount of solvent stock solution (prepared in step 3) required to obtain a concentration of 10 mg/ml.

For example, if you weigh out 4.2 mg DHB, dissolve in 420 μ l solvent stock solution.

5. Dilute the matrix solution by a factor of ten. Pipet 35 μ l of the matrix solution prepared in step 4 into a clean microcentrifuge tube containing 315 μ l of solvent stock solution (prepared in step 3). Mix by vortexing.

CHCA Matrix Working Solution (0.1 mg/ml in 84:13:3 acetonitrile : ethanol : 5 mM ammonium citrate in 0.1% TFA)

1. Prepare a 50 mM (= 11.3 mg/ml) ammonium citrate (dibasic) solution in 0.1 % TFA in a 1.5 ml microcentrifuge tube. Mix by vortexing.
2. Dilute the solution prepared in step 1 by a factor of ten. Pipet 100 μ l of 50 mM ammonium citrate into 900 μ l 0.1% TFA and mix by vortexing.
3. Make a solvent stock solution of 84% (v/v) acetonitrile: 13% (v/v) ethanol: 3% (v/v) 5 mM ammonium citrate in 0.1% TFA. Pipet 1000 μ l acetonitrile, 155 μ l ethanol, and 36 μ l 5 mM ammonium citrate in 0.1% TFA (prepared in step 2) into a 1.5 ml microcentrifuge tube and mix by vortexing.

- 4. Weigh out 3–8 mg CHCA into an empty Matrix Mixing Vial and dissolve in the amount of solvent stock solution (prepared in step 3) required to obtain a concentration of 10 mg/ml.**

For example, if you weigh out 4.2 mg CHCA, dissolve in 420 μ l solvent stock solution.

- 5. Dilute the matrix solution 1 in 100 to a concentration of 0.1 mg/ml. Pipet 10 μ l of the matrix solution prepared in step 4 into a clean microcentrifuge tube containing 990 μ l of solvent stock solution (prepared in step 3). Mix by vortexing.**

Matrix Solution Reagent Summary

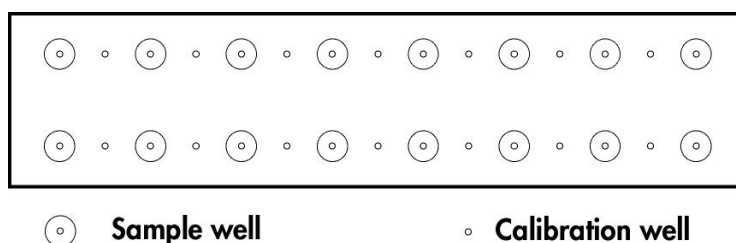
| | |
|--|--|
| 50 mM ammonium citrate (dibasic) in 0.1 % TFA | 11.3 mg ammonium citrate (dibasic) per ml 0.1 % TFA |
| Solvent stock solution | 1000 μ l acetonitrile; 155 μ l ethanol; 36 μ l 5 mM ammonium citrate (dibasic) in 0.1% TFA |
| DHB matrix stock solution | 10 mg/ml DHB in solvent stock solution |
| DHB matrix working solution | 35 μ l DHB matrix stock solution; 315 μ l solvent stock solution |
| CHCA matrix stock solution | 10 mg/ml CHCA in solvent stock solution |
| CHCA matrix working solution | 10 μ l CHCA stock solution; 990 μ l solvent stock solution |

Appendix E: Mass·Spec·Focus Chip Geometries*

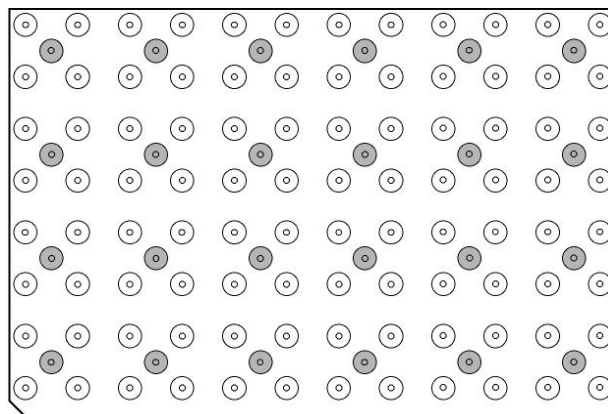
The laminated pipetting guide supplied with Chip Types 1 and 2 helps to locate wells. Chip Types 3 and 4 have etched guidelines. Chip Types 5 and 6 have etched guide spots between the sample wells to aid pipetting (see below).

In addition to sample wells, Mass·Spec·Focus Chip types 1, 2, 5 and 6 contain calibration wells. On type 1, 5, and 6 chips, the calibration wells contain only an Analysis Zone. Type 2 chip calibration wells have the same functionality (i.e., desalting or purification) as sample wells.

Type 1 Chips for Shimadzu Kratos MALDI-MS instruments (cat. nos. 49201, 49300, and 49400) 2 x 8 sample wells + 14 calibration wells

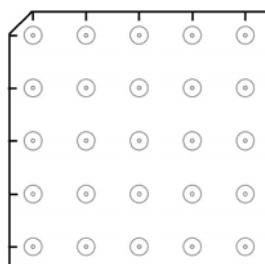


Type 2 Chips for Waters MALDI-MS instruments (cat. nos. 49202, 49301, and 49401) 12 x 8 sample wells + 24 calibration wells (grey)

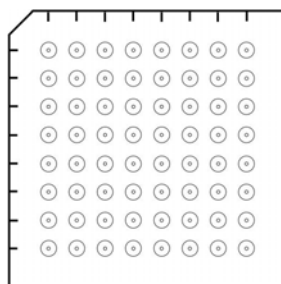


* Chips are not drawn to scale

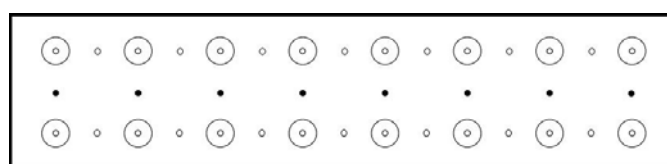
Type 3 Chips or Applied Biosystems MALDI-MS instruments (cat. nos. 49203, 49302, and 49402) 5 x 5 sample wells



Type 4 Chips for Applied Biosystems MALDI-MS instruments (cat. nos. 49204, 49303, and 49403) 8 x 8 sample wells

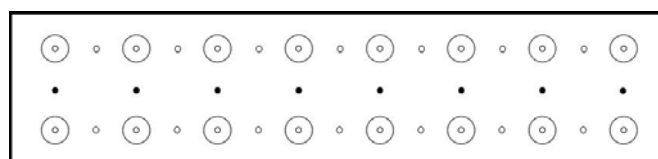


Type 5 Chips for Thermo Electron MALDI-MS instruments (cat. nos. 49205, 49304, and 49404) 2 x 8 sample wells + 14 calibration wells



○ Sample well ◦ Calibration well • Guide spot

Type 6 Chips for Bruker Daltonics MALDI-MS instruments (cat. nos. 49206, 49305, and 49405) 2 x 8 sample wells + 14 calibration wells



○ Sample well ◦ Calibration well • Guide spot

Appendix F: Loading and Running Mass·Spec·Focus Chips on MALDI Mass Spectrometers

Mass·Spec·Focus and Mass·Spec·Focus Desalting Chips are available for a wide range of different MALDI mass spectrometers (see Ordering Information, page 37). To run the chips on a particular mass spectrometer the chip has to be placed in a special adapter or customized holder. These holders are available either from the original instrument manufacturer or directly from QIAGEN (depending on the instrument). You will find a comprehensive overview including ordering information for the adapter, interface, or holder that will match your MALDI instrument on the QIAGEN mass spectrometry web page (www.qiagen.com/maldiprep).

For Mass·Spec·Focus Chips types 2, 3, and 4, only one chip can be placed in the corresponding holder. For Mass·Spec·Focus Chips types 1, 5, and 6, up to six chips can be placed in a sample holder. If the user wishes to use less than the maximum 6 chips in the holder, then the remaining positions need to have blanks loaded in the slots instead of chips. The blanks are reusable and should be stored after the experiment for future use.

Additionally the actual plate layout of the chip, which can differ for different MS instruments, has to be correctly set in the instrument software. This is typically done either by choosing a predefined plate layout in the software or by importing the corresponding plate or geometry file. You will also find detailed instructions on how to set the correct plate layout in your MALDI instrument software also on the QIAGEN mass spectrometry web page (www.qiagen.com/maldiprep).

Ordering Information

| Product | Contents | Cat. no. |
|---|--|----------|
| Mass·Spec·Focus Chip Type 1 | For Shimadzu Kratos MALDI-MS instruments: 6 chips with 16 wells for on-chip concentration of MALDI samples | 49201 |
| Mass·Spec·Focus Chip Type 2 | For Waters MALDI-MS instruments: 1 chip with 96 wells for on-chip concentration of MALDI samples | 49202 |
| Mass·Spec·Focus Chip Type 3 | For Applied Biosystems MALDI-MS instruments: 1 chip with 25 wells for on-chip concentration of MALDI samples | 49203 |
| Mass·Spec·Focus Chip Type 4 | For Applied Biosystems MALDI-MS instruments: 1 chip with 64 wells for on-chip concentration of MALDI samples | 49204 |
| Mass·Spec·Focus Chip Type 5 | For Thermo Electron MALDI-MS instruments: 6 chips with 16 wells for on-chip concentration of MALDI samples | 49205 |
| Mass·Spec·Focus Chip Type 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 Type 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 Type 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 Type 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 Type 4 | For Applied Biosystems MALDI-MS instruments: 1 chip with 64 wells for on-chip cleanup and concentration of MALDI samples | 49303 |
| Mass·Spec·Focus Desalting Chip Type 5 | For Thermo Electron MALDI-MS instruments: 6 chips with 16 wells for on-chip cleanup and concentration of MALDI samples | 49304 |

| Product | Contents | Cat. no. |
|---------------------------------------|--|----------|
| Mass·Spec·Focus Desalting Chip Type 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 Chip Solvent Kit | For 1000 MALDI sample preparations: acetonitrile (3 x 10 ml), ethanol (5 x 2 ml), 0.1% TFA (3 x 10 ml), CHCA (4 x 25 mg), DHB (4 x 25 mg), ammonium citrate (3 x 50 mg), peptide standard, tubes, mixing vials | 49200 |
| Mass·Spec·Focus Humidity Chamber | Controlled humidity chamber for Mass·Spec·Focus Chips | 49903 |

Mass·Spec·Turbo Chips — pre-spotted matrix chips for high-throughput MALDI analysis

| | | |
|--|--|-------|
| Mass·Spec·Turbo 192 Peptide Chip* Kit Type 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* Kit Type 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 192 Protein Chip Kit Type 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 Kit Type 1 | 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 |
| Probot Table (9 Chips) | Multichip holder for automated sample loading using the Probot Microfraction Collector | 49902 |

* Also available in 625- and 1600-spot formats; please inquire.

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Austria ■ Orders 0800/28-10-10 ■ Fax 0800/28-10-19 ■ Technical 0800/28-10-11

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

Canada ■ Orders 800-572-9613 ■ Fax 800-713-5951 ■ Technical 800-DNA-PREP (800-362-7737)

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

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

Finland ■ Orders 0800-914416 ■ Fax 0800-914415 ■ Technical 0800-914413

France ■ Orders 01-60-920-920 ■ Fax 01-60-920-925 ■ Technical 01-60-920-930

Germany ■ Orders 02103-29-12000 ■ Fax 02103-29-22000 ■ Technical 02103-29-12400

Ireland ■ Orders 1800 555 049 ■ Fax 1800 555 048 ■ Technical 1800 555 061

Italy ■ Orders 02-33430411 ■ Fax 02-33430426 ■ Technical 800 787980

Japan ■ Telephone 03-5547-0811 ■ Fax 03-5547-0818 ■ Technical 03-5547-0811

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

USA ■ Orders 800-426-8157 ■ Fax 800-718-2056 ■ Technical 800-DNA-PREP (800-362-7737)

