Instructions for Use

Profiling Kit MB-WCX

As part of Bruker Daltonics ClinProt™ system the Profiling Kits (based on the magnetic bead technology utilizing different interaction mechanisms such as reverse phase, cation exchange or affinity to immobilized copper ions) are designed for sophisticated biomarker profiling studies on a discovery level. Combining respective dedicated bead functionalities allow systematic and intelligent biomarker discovery workflows within a huge mass range from 1000 Da up to 70,000 Da.
General Information

Proteome and peptidome profiling of biofluids is a promising approach in the field of biomarker discovery and proteomic pattern diagnostic. Current challenge is the enrichment of respective molecules out of complex samples like serum, plasma and urine. For a successful biomarker search smart as well as intelligent techniques have to be applied.

Bruker Daltonics provides the mass spectrometry based ClinProt™ system solution for preparation, measurement and visualization of peptides and proteins in the context of clinical proteomics. This system consists of dedicated sample preparation tools, superior instruments for mass spectrometric analyses, and an outstanding bioinformatics package.

Sample preparation with Bruker Daltonics ClinProt™ system is based on the magnetic bead technology. Peptides and proteins are extracted from complex biological material and concentrated on the surface of magnetic microparticles. Eluted molecules are spotted directly to a MALDI-TOF target without further purification steps. All sample processing steps can be performed manually, using a manual magnet separator, or fully automated using suitable robotic systems for higher sample throughput. Mass spectra acquisition is done by Bruker Daltonics’ MALDI-TOF mass spectrometers (e.g. microflex®, autoflex® or ultraflex®). The software package ClinProTools™ allows visualization and comparison of large data sets, statistical peak evaluation, pattern recognition and thus discovery of biomarker candidates.

Applying different bead functionalities allows the enrichment of different peptides and proteins, thus increasing the chance to find a biomarker of value. Three different bead functionalities (MB-HIC 8, MB-WCX, MB-IMAC Cu) are available as sample preparation tools in a profiling approach on a discovery level. Isolation and concentration using MB-HIC 8 is based on reverse phase interaction, whereas MB-WCX is a weak cation exchanger and MB-IMAC Cu depends on metal ion affinity. The handling of the magnetic beads technology is simple and can be easily scaled-up and automated even if only small sample volumes are available.

Successful applications and reproducibility of the MB-WCX beads using serum, plasma and urine samples is demonstrated in various studies [1-6].
Intended Use

The Profiling Kit MB-WCX (Magnetic Beads based Weak Cation Exchange Chromatography) is developed for the enrichment of proteins and peptides from biological samples based on cation exchange chromatography prior to MALDI-TOF mass spectrometry analysis by means of e.g. Bruker’s microflex®, autoflex® or ultraflex® MALDI-TOF.

This kit is for research use only. It is not for use in diagnostic procedures.

Kit components

<table>
<thead>
<tr>
<th>Materials provided in the kits</th>
<th>Abbreviation</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnetic Beads MB-WCX</td>
<td>MB</td>
<td></td>
</tr>
<tr>
<td>MB-WCX Binding Buffer</td>
<td>BB</td>
<td></td>
</tr>
<tr>
<td>MB-WCX Wash Buffer</td>
<td>WB</td>
<td></td>
</tr>
<tr>
<td>MB-WCX Elution Buffer</td>
<td>EB</td>
<td></td>
</tr>
<tr>
<td>MB-WCX Stabilization Buffer</td>
<td>SB</td>
<td>Irritant</td>
</tr>
</tbody>
</table>

Ordering information

<table>
<thead>
<tr>
<th>Product</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Profiling Kit 100 MB-WCX</td>
<td>8223983</td>
</tr>
</tbody>
</table>

Storage and stability

Kit components should be stored and handled carefully to avoid contaminations. Kits are shipped at ambient temperature. We recommend storing the kit at 2 - 8 °C after arrival. Do not freeze!

If stored unopened under above conditions, the product is stable until expiration date mentioned on the label.

Please note:

After the first opening, period of storage should not exceed three months. For your convenience, we provide the possibility to mark the respective expiry date on the vial’s label. After usage, keep the vials closed. A drying up of the beads will damage the product irreversible!
Risk and safety information

The kit has to be labeled due to the calculation procedure of the "General Classification guideline for preparations of the EU" in the latest valid version. Any national or local safety regulations have to be observed. This kit contains an irritant component. Please read and observe the Material Safety Data Sheet which is available for download at www.bruker.com/msds in the product description area.

Beside the kit components, we recommend further chemicals within these Instructions for Use. Please read and observe the respective Material Safety Data Sheet to be provided by your supplier. Observe the general safety regulations when handling chemicals.

Workflow for clinical sample preparation

Biomarker discovery studies and proteomic pattern diagnostic applications require outstanding reproducible and standardized procedures as well as sophisticated tools. Thus, as fundamental prerequisites it is absolutely necessary to work in a clean environment, using suitable plastics and clean MALDI-MS targets as well as operating a well maintained mass spectrometer (for details pay attention to the following recommended preparation procedures). For a closed profiling study, the use of kits from one lot only maximizes the comparableness of the samples within the study.

Fig. 1: Sample preparation workflow comprising the magnetic bead based separation technology, MALDI-MS target preparation and mass spectrometric spectra acquisition as well as subsequent analysis.
Aim of clinical study

Considering the respective purpose of a current study it is absolutely necessary to generate suitable circumstances in advance (cf. Tab. 1). Following categories of study’s aims might occur:

**Table 1:** Different aims of clinical relevant studies and recommended required prerequisites.

<table>
<thead>
<tr>
<th>Aim of study</th>
<th>Grade of reproducibility</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proof of principle study</td>
<td>Intra-laboratory</td>
<td>(1) Samples: Small sample cohorts</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2) Kind of preparation: manual</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3) Number of mass spectrometers: one</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4) Number of operators: one</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(5) Time period of measurements: one day</td>
</tr>
<tr>
<td>Biomarker search</td>
<td>Intra-laboratory and</td>
<td>(1) Samples: Hundreds of standardized samples</td>
</tr>
<tr>
<td></td>
<td>Inter-day</td>
<td>(2) Kind of preparation: automatic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3) Number of mass spectrometers: one</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4) Number of operators: several</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(5) Time period of measurements: several weeks until few months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(6) Laboratory conditions: Air-conditioned</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(7) Usage of a well-maintained and well-tuned mass spectrometer utilizing a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>well-defined standard (e.g. Bruker Daltonics' ClinProt Standard)</td>
</tr>
<tr>
<td>Diagnostic application*</td>
<td>Intra-laboratory</td>
<td>(1) Samples: Hundreds of standardized samples</td>
</tr>
<tr>
<td></td>
<td>Inter-day</td>
<td>(2) Kind of preparation: automatic</td>
</tr>
<tr>
<td></td>
<td>Inter-laboratory</td>
<td>(3) Number of mass spectrometers: several in different laboratories</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4) Number of operators: several</td>
</tr>
<tr>
<td></td>
<td>Inter-laboratory</td>
<td>(5) Time period for measurements: years</td>
</tr>
<tr>
<td></td>
<td>Inter-laboratory</td>
<td>(6) Laboratory conditions: Air-conditioned</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(7) Usage of a well-maintained and well-tuned mass spectrometer utilizing a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>well-defined standard</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(8) Adaptation of analytical performance of several mass spectrometers in</td>
</tr>
<tr>
<td></td>
<td></td>
<td>different laboratories (cf. Bruker Daltonics &quot;Manual for Standardized ClinProt</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Measurements&quot; at <a href="http://www.bruker.com/care">www.bruker.com/care</a>: Profiling Kits)</td>
</tr>
</tbody>
</table>

*Note: This kit is for research use only. It is not for use in diagnostic procedures. We recommend the use of kits from one lot only to maximize the reproducibility.
Please note following general remarks:

Be cautious with the samples you would like to use within your studies. Not all storage conditions or collection procedures are suitable for subsequent mass spectrometry based clinical research. For usage of serum samples or urine samples follow the recommendations of Baumann et al. [7] and Fiedler et al. [4].

Use solvents with HPLC grade or better!

Many plastics are not compatible with the subsequent matrix preparation on AnchorChip™ or mass spectrometric analysis in general because they release polymers. Avoid all kind of siliconized tubes! Problems with unsuitable plastics typically result in strange looking crystallisation and polymer signals. In extreme cases samples do not crystallize at all and remain liquid even after hours. Use recommended plastics of proven MALDI-TOF MS analysis compatible brands (see ordering information).

For MALDI-TOF MS analysis it is necessary to work in a very clean environment! Every air pollution will definitely impair the spectrum quality.

Following conditions are recommended for an optimal sample crystallisation on the MALDI target:

- Operating temperature: 5-40 °C (41-104°F)
- Operating humidity: 25%-65% at 22°C
- Atmospheric pressure: 75-105 kPa

Regular cleaning of the ion source is recommended and will yield spectra with lower noise and better resolved peaks. For this procedure Bruker Daltonics’ source shower target can be used regularly [see ordering information]. For a complete cleaning of the ion source please ask Bruker Daltonics’ service team (maldi.support@bruker.com).
Recommended BioMarker separation procedure

Generally, we recommend the following protocol for sample preparation (Fig. 2) but, in dependence on the kind of sample and application, it might be necessary to adapt this protocol. For this purpose, different parameters (e.g. applied bead amount, volumes of sample and binding solution, volume of elution buffer) can be varied. Further fractionation of samples can be achieved by substitution of binding buffer or elution buffer. But applicable alternative buffers have to be found out empirically.

1. Binding  
2. Washing  
3. Elution

Fig. 2: Magnetic bead based sample preparation workflow
Binding the sample to the magnetic beads

1. Mix the magnetic beads thoroughly on a vortex device for 1 minute.

2. Transfer 10 µL BB and 10 µL MB-WCX beads to a standard thin wall PCR-tube and mix by pipetting up and down.

3. Add 5 µL serum to the solution and mix intensively by pipetting up and down five times.

4. Wait five minutes for incubation.

5. Place the tube into the magnetic separator and collect the beads at the wall of the tube for 1 minute. The supernatant should be clear.

6. Remove supernatant carefully by using a pipette. Avoid contact of pipette tips with the beads and take care not to remove beads.

Washing of the sample bound to the magnetic beads

7. Add 100 µL WB to the tube.
8. Move the tube back and forth in the magnetic separator ten times and note the movement of the beads.

9. Collect the beads at the tube wall for 1 minute.

10. Remove supernatant carefully by using a pipette.

11. Repeat steps 7-10 twice.

Eluting the sample bound to the magnetic beads

12. Add 5 µL EB and dissolve the beads from the tube wall by pipetting up and down intensively 10 times.

13. Collect the beads at the tube wall for 2 minutes.

14. Transfer the clear supernatant into a fresh tube.

15. Add 5 µL SB to the eluate and mix intensively by pipetting up and down.

⚠️: SB is irritant (H: 315, 319; P: 280, 305+351+338, 321, 362, 332+313, 337+313)
16. Proceed with the appropriate MALDI-TOF target preparation protocol as described below or store your sample appropriately.

Adapted preparation protocol for urine samples according to Fiedler et al. [4]

Step 2 has to be modified as follows: 30 µL urine sample (protein concentration: 8-140 mg/L) was diluted in 60 µL MB-WCX binding solution and 10 µL WCX beads were added. After thorough stirring, sample mixtures were incubated for 1 minute at room temperature. Afterwards, the preparation protocol was continued as described in the standard preparation protocol.

Note: Interindividual variation in urine patterns can be minimized by equalization of the total protein content. To obtain equalized samples, Fiedler et al. repeated the loading procedure from step 2 after removal of the unbound supernatant. However, this procedure is hard to automate. Therefore, a preconcentration of urine samples followed by equalization of the protein or creatinine content is recommended for automatic sample preparation using a robotic system.

MALDI-TOF MS target preparation

MALDI-TOF target preparation is one of the most critical steps related to high quality and reproducible MS spectra. The below described workflow is strongly recommended. Always start with a thoroughly cleaning of the respectively MALDI-MS target of choice. Subjected to the sample’s characteristics choose the suitable MALDI-Matrix (e.g. α-cyano-4-hydroxycinnamic acid (HCCA) for peptides or 2,5-dihydroxyacetophenone (DHAP) for proteins) and follow the respective sample preparation steps.

Always use an absolute clean MALDI-MS target or AnchorChip™.

The following MALDI target, BigAnchor™ and AnchorChip™ cleaning protocols differ significantly from standard procedures recommended for Bruker Daltonics’ targets and AnchorChips™.
### Cleaning of MALDI-MS targets

**Cleaning of polished steel targets**
This is an advanced cleaning protocol differing from the standard cleaning procedure recommended in the respective target’s product description.

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Rinse the target intensively under flowing hot tap water.</td>
</tr>
</tbody>
</table>
| 2.   | Wipe the target intensively with acetone using a kimwipe.  

⚠️ **Acetone is highly flammable and irritant (H: 225, 319, 336; P: 210, 261, 305+351+338)**  

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.</td>
<td>Rinse the target intensively under flowing hot tap water.</td>
</tr>
</tbody>
</table>
| 4.   | Wipe the target intensively with 80% TFA using a kimwipe.  

⚠️ **80% TFA is corrosive (H: 314, 332, 412; P: 261, 273, 280, 303+361+353, 304+340+310, 305+351+338)**  

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.</td>
<td>Rinse the target intensively under flowing hot tap water.</td>
</tr>
<tr>
<td>6.</td>
<td>Rinse the target with distilled water (Milli-Q).</td>
</tr>
</tbody>
</table>
| 7.   | Rinse the target with methanol and let it dry.  

⚠️ **Methanol is highly flammable and toxic (H: 225, 301+311+331, 370; P: 210, 260, 280, 301+310+311)**
**Cleaning of AnchorChip™ and BigAnchor™ targets**

This is an advanced cleaning protocol differing from the standard cleaning procedure recommended in the respective target's product description.

1. Rinse the target intensively under flowing hot tap water.  
   **Hot tap water**

2. Wipe the target intensively with acetone using a kimwipe.  
   **Acetone**  
   : Acetone is highly flammable and irritant (H: 225, 319, 336; P: 210, 261, 305+351+338)

3. Rinse the target with distilled water (Milli-Q).  
   **Distilled water**

4. Rinse the target with methanol and let it dry  
   **Methanol**  
   : Methanol is highly flammable and toxic (H: 225, 301+311+331, 370; P: 210, 260, 280, 301+310+311)

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**Matrix preparation on the MALDI-MS targets**

**Usage of HCCA for profiling experiments on polished steel targets**

Recommended mass range: 1-10 kDa  
Matrix: HCCA  
Target: MTP 384 target plate polished steel, MTP 384 target plate ground steel, MSP target polished steel or MSP 96 target ground steel

1. Apply 1 µL of sample to a target spot and let it dry at room temperature (3-10 min, not longer, oxidation!)  
   **1 µL Sample**
2. Apply 1 µL of matrix consisting of HCCA (3 mg/mL) in 50% acetonitrile/2% TFA

⚠️ HCCA is harmful (H: 315, 319, 335; P: 261, 280, 305+351+338, 321, 405, 501), acetonitrile is highly flammable and harmful (H: 225, 302, 312+332, 319; P: 210, 280, 305+351+338), 2% TFA is irritant (H: 315, 319; P: 264, 280, 302+352, 305+351+338, 321, 332+313, 337+313, 362)

1 µL Matrix Solution

3. Let it dry at room temperature.

Usage of HCCA for profiling experiments on BigAnchor™ targets (manual preparation technique only)

Recommended mass range: 1-10 kDa
Matrix: HCCA
Target: MTP BigAnchor™ 384 or MSP BigAnchor™ 96

1. Suspend 15 mg HCCA in 250 µL acetonitrile and add 250 µL 2% aqueous trifluoroacetic acid; vortex the suspension for 1 min at room temperature.

⚠️ HCCA is harmful (H: 315, 319, 335; P: 261, 280, 305+351+338, 321, 405, 501), acetonitrile is highly flammable and harmful (H: 225, 302, 312+332, 319; P: 210, 280, 305+351+338), 2% TFA is irritant (H: 315, 319; P: 264, 280, 302+352, 305+351+338, 321, 332+313, 337+313, 362)

15 mg HCCA
250 µL ACN
250 µL 2% TFA
Vortex

2. Sonicate the suspension for 10 min.

Sonicate

3. Centrifuge the saturated solution for 2 min at 13000 rpm; use the supernatant for MALDI TOF MS measurements.

Centrifuge
4. Dilute 1 µL of (eluted) sample in 10 µL of matrix solution and mix the solution by pipetting up and down for at least 5 times.

| 1 µL Sample solution |
| 10 µL Matrix Solution |

5. Place 1 µL of the analyte/matrix solution on the BigAnchor of the target and let it dry at room temperature.

| 1 µL of Sample/Matrix Solution |

Usage of HCCA for profiling experiments on AnchorChip™ targets

Recommended mass range: 1-10 kDa
Matrix: HCCA
Target: MTP AnchorChip™ 600/384 or MSP AnchorChip™ 600/96

1. Prepare a matrix solution with HCCA of 0.3 g/L in ethanol:acetone 2:1 (prepare matrix solution daily fresh).

⚠️: HCCA is harmful (H: 315, 319, 335; P: 261, 280, 305+351+338, 321, 405, 501), ethanol is highly flammable (H: 225, 319; P: 210, 280, 305+351+338, 337+313, 403+235), acetone is highly flammable and irritant (H: 225, 319, 336; P: 210, 261, 305+351+338)

2. Dilute 1 µL of (eluted) sample in 10 µL of matrix solution.

3. Spot 1 µL of this solution to a 600 µm diameter AnchorChip™ target and let it air-dry.

| 1 µL of Sample/Matrix Solution |
Usage of 2,5-DHAP for profiling experiments on BigAnchor and steel targets (manual preparation technique only)

Recommended mass range: 6-20 kDa & 20-100 kDa
Matrix: 2,5-DHAP
Target: MTP BigAnchor™ 384, MSP BigAnchor™ 96, MTP 384 target plate polished steel, MTP 384 target plate ground steel, MSP 96 target polished steel or MSP 96 target ground steel

1. Suspend 7.6 mg 2,5-DHAP in 375 µL ethanol.
   - 2,5-DHAP is irritant (H: 315, 319, 335; P: 261, 305+351+338), ethanol is highly flammable (H: 225, 319; P: 210, 280, 305+351+338, 337+313, 403+235)

2. Add 125 µL (10 µmol) of a diammonium hydrogen citrate solution (18 mg/mL in Milli-Q water).
   - Diammonium hydrogen citrate is irritant (H: 319, 335; P: 261, 305+351+338)

3. Vortex for 1 min at room temperature.

4. Sonicate for 15 min followed by vortexing for 1 min at room temperature. (The matrix solution is stable for one week if stored in the dark at room temperature).

5. Mix 2 µL of (eluted) sample with 2 µL of 2% TFA, then add 2 µL of the 2,5-DHAP matrix solution and mix vigorously by pipetting up and down until the liquid gets cloudy.
   - 2% TFA is irritant (H: 315, 319; P: 264, 280, 302+352, 305+351+338, 321, 332+313, 337+313, 362)
6. Spot 1 µL of the mixture on the BigAnchor of the target and let it dry at room temperature.

Usage of 2,5-DHAP for profiling experiments on AnchorChip targets

Recommended mass range: 6-20 kDa & 20-100 kDa
Matrix: 2,5-DHAP
Target: MTP AnchorChip™ 600/384 or MSP AnchorChip™ 600/96

1. Suspend 7.6 mg 2,5-DHAP in 375 µL ethanol.

   : 2,5-DHAP is irritant (H: 315, 319, 335; P: 261, 305+351+338), ethanol is highly flammable (H: 225, 319; P: 210, 280, 305+351+338, 337+313, 403+235)

2. Add 125 µL (10 µmol) of a diammonium hydrogen citrate solution (18 mg/mL in Milli-Q water).

   : Diammonium hydrogen citrate is irritant (H: 319, 335; P: 261, 305+351+338)

3. Vortex for 1 min at room temperature.

4. Sonicate for 15 min followed by vortexing for 1 min at RT.

5. Store the matrix solution in the dark! (It is stable for one week.)
6. Mix 2 µL of (eluted) sample with 2 µL of 2% TFA, then add 2 µL of the 2,5-DHAP matrix solution.

![Warning: 2% TFA is irritant (H: 315, 319; P: 264, 280, 302+352, 305+351+338, 321, 332+313, 337+313, 362)]

7. The matrix/analyte mixture should be vigorously mixed.

8. Spot 0.5 - 1 µL of this mixture on the Anchor of the target, prevent air-bubbles.

MALDI-TOF MS Spectra Acquisition

Before starting the MS analysis the FlexControl method to be used has to be calibrated and if necessary optimized. Bruker’s MALDI-TOF mass spectrometer are equipped with a number of default acquisition methods which cover different mass ranges and it is recommended for inexperienced users to start working with one of them and to adapt the method to their current approaches. For profiling experiments in the mass range 1 - 10 kDa, we recommend to use the flexControl method “LP_Clinprot” as starting point. For optimum performance of the instrument prepare the ClinProt Standard (CPS, see below) on a target and check the resolution values. All changes must be saved before starting an automatic run.
Setting up the mass spectrometer

Bruker Daltonics recommends utilizing the ClinProt Standard CPS as a standard sample

**Material:**
- 0.1 % TFA
- 100 % Acetone
- 100 % Ethanol
- 10 mM Ammonium acetate
- Peptide calibration standard
- Protein calibration standard I

**CPS preparation procedure**

1. **Solubilize the Peptide Calibration Standard (one vial) in 125 µL of 0.1% TFA for 5 min at room temperature and vortex for 1 min.**

2. **Solubilize the Protein Calibration Standard I (one vial) in 125 µL of 0.1% TFA for 5 min at room temperature and vortex for 1 min.**

3. **Prepare a 10 mM ammonium acetate solution (770 mg/L ammonium acetate in Milli-Q water.**

4. **Mix the CPS standard regarding the following scheme:**
   - 5 µL Peptide Calibration Standard
   - 20 µL 10mM ammonium acetate
   - 25 µL Protein Calibration Standard I

5. **Mix well for 1 min by vortexing, use fresh (or store at 4°C for some days or store in aliquots at -20°C for at least one week).**
**Preparation of CPS on AnchorChip™ or polished steel target**

**AnchorChip™ target**

<table>
<thead>
<tr>
<th>Step</th>
<th>Instruction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Thaw an aliquot of CPS (approx. 10 µL) at room temperature.</td>
</tr>
<tr>
<td>2.</td>
<td>Mix 1 µL of CPS with 10 µL of freshly prepared HCCA matrix solution.</td>
</tr>
<tr>
<td>3.</td>
<td>Pipette 0.5-1 µL of CPS/Matrix mixture on different calibrant positions of a 600 µm AnchorChip target and let it dry at room temperature.</td>
</tr>
</tbody>
</table>

**BigAnchor™ target**

<table>
<thead>
<tr>
<th>Step</th>
<th>Instruction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Thaw an aliquot of CPS (approx. 10 µL) at room temperature.</td>
</tr>
<tr>
<td>2.</td>
<td>Dilute 1 µL of CPS in 10 µL of freshly prepared HCCA matrix solution and mix the solution by pipetting up and down for at least 5 times.</td>
</tr>
<tr>
<td>3.</td>
<td>Place 1 µL of the analyte/matrix solution on the BigAnchor of the target and let it dry at room temperature.</td>
</tr>
</tbody>
</table>
Steel targets

Use the space between target spots for preparation of the CPS to prevent memory effects!

1. Thaw an aliquot of CPS (approx. 10 µL) at room temperature.

2. Pipette 1 µL CPS on different positions of the target and let it dry for few minutes (3-10 min) at room temperature.  
   1 µL CPS

3. Pipette 1 µL HCCA matrix solution on the same spots and let it dry at room temperature, too.  
   1 µL HCCA Matrix Solution

Spectra generation using CPS

1. Introduce the AnchorChip™ or polished steel target into the mass spectrometry instrument and choose the appropriate linear FlexControl™ method “LP_Clinprot”.

2. To generate a CPS spectrum vary the laser power.

3. Use 10 laser shots with approx. 10-15% higher laser power as the final power = “matrix blaster”.

4. After matrix blaster take 30 shots of the laser power that is slightly above the desorption threshold. The maximum intensity values of the peaks should be between 1000 and 3000. Add only these shots into the sum buffer.
5. Repeat spectrum acquisition in the same manner from 5-10 different positions to get in total 150 shots (5 × 30). An exemplary CPS spectrum is displayed in Fig. 3.

**Fig. 3:** ClinProt Standard in linear mode - *ultraflex*®
**Calibration using CPS**

1. After accumulation of 150 shots estimate the intensity values for all peaks within the sum buffer. All peak intensities must be beyond the absolute intensity threshold value set.

2. Use the sum buffer for calibration.

3. Take the masses from the reference list “Clinprot_standard”.

4. Try to include all peptides/proteins from the CPS and use a quadratic fit.

5. Save the calibration to the FlexControl™ method.

**Check of resolution values**

1. Use the same sum spectrum for checking resolution values as for calibration.

2. Use “Maximum cursor left” and “Show peak information” for displaying resolution values of every peak.

3. Resolution values described in Table 2 should be reached at minimum.
**Table 2:** Constitution of the ClinProt Standard

<table>
<thead>
<tr>
<th>Substance</th>
<th>Average Mass</th>
<th>Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiotensin II (M+H)$^+$</td>
<td>1047.19</td>
<td>≥ 350</td>
</tr>
<tr>
<td>Angiotensin I (M+H)$^+$</td>
<td>1297.49</td>
<td>≥ 355</td>
</tr>
<tr>
<td>Substance P (M+H)$^+$</td>
<td>1348.64</td>
<td>≥ 365</td>
</tr>
<tr>
<td>Bombesin (M+H)$^+$</td>
<td>1620.86</td>
<td>≥ 400</td>
</tr>
<tr>
<td>ACTH clip 1-17 (M+H)$^+$</td>
<td>2094.43</td>
<td>≥ 450</td>
</tr>
<tr>
<td>ACTH clip 18-39 (M+H)$^+$</td>
<td>2466.68</td>
<td>≥ 520</td>
</tr>
<tr>
<td>Somatostatin 28 (M+H)$^+$</td>
<td>3149.57</td>
<td>≥ 555</td>
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<tr>
<td>Ubiquitin (M+2H)$^{2+}$</td>
<td>4283.39</td>
<td>≥ 760</td>
</tr>
<tr>
<td>Insulin (M+H)$^+$</td>
<td>5734.51</td>
<td>≥ 555</td>
</tr>
<tr>
<td>Cytochrome c (M+2H)$^{2+}$</td>
<td>6180.49</td>
<td>≥ 475</td>
</tr>
<tr>
<td>Ubiquitin (M+H)$^+$</td>
<td>8565.76</td>
<td>≥ 320</td>
</tr>
</tbody>
</table>
Workflow of measurement of serum samples

1. Check the crystallization of the spot to be analyzed.

   An exemplary spot is shown in Fig. 4.

2. Find out the laser power slightly above the desorption level.

3. Find out the laser power and the number of shots for matrix blaster.

   For matrix blaster use approx. 10-20 laser shots with approx. 10-20% higher laser power as the final power (slightly above the desorption level). After the matrix blaster only top of peaks should be visible (cf. Fig. 5).

4. Tune the final laser power.

   Make sure, that the intensity of the highest peak does not come to saturation. After accumulation of e.g. 30 shots the intensity of the highest peak should be preferably higher than 1000 [arb] (Fig. 6).

5. Finally accumulate at minimum 450 (15 × 30) shots from 15 different positions (Fig. 7).

   The more shots will be accumulated the merrier are the S/N ratio and the reproducibility of the obtained sum spectrum.
**Fig. 4:** Exemplary crystallization of a sample/HCCA mixture on polished steel target after processing of 5 µL human serum using MB-WCX.

**Fig. 5:** Single spectrum after accumulation of 40 shots slightly above the desorption level.
Fig. 6: Baseline subtracted sum spectrum of a serum sample (1-10 kDa) using HCCA as matrix after accumulation of 1000 shots (40 shots each from 25 different positions on the same spot): Spectrum acquisition was performed at 20 Hz repetition rate on a microflex® mass spectrometer.
References


<table>
<thead>
<tr>
<th>Problem</th>
<th>Reason</th>
<th>What to do</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strange looking crystallization/no crystallization at all</td>
<td>Unsuitable tips, tubes or flasks.</td>
<td>Cf. list of recommended MALDI compatible plastics</td>
</tr>
<tr>
<td>Strange looking crystallization/no crystallization at all</td>
<td>Insufficient grade of purity of the solvent</td>
<td>Cf. list of recommended MALDI compatible solvents</td>
</tr>
<tr>
<td>Strange looking crystallization/no crystallization at all</td>
<td>Too high/low temperature and/or humidity</td>
<td>Cf. recommended environmental conditions</td>
</tr>
<tr>
<td>Strange looking crystallization/no crystallization at all</td>
<td>Detergents in the sample</td>
<td>Remove detergent before magnetic bead-based sample preparation. Possibly, exchange the bead functionality.</td>
</tr>
<tr>
<td>Strange looking crystallization/no crystallization at all</td>
<td>Amount of lipids in the sample to high</td>
<td>Standard preparation protocol inapplicable: Try different amount of sample and/or bead volume</td>
</tr>
<tr>
<td>Strange looking crystallization/no crystallization at all</td>
<td>Ultra-sonic cleaning of the AnchorChip target</td>
<td>Respective AnchorChip target is definitely useless for bead-based sample preparations</td>
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<tr>
<td>Low S/N ratio and few peaks</td>
<td>Bad environmental conditions for crystallization</td>
<td>See above</td>
</tr>
<tr>
<td>Low S/N ratio and few peaks</td>
<td>Air pollution (Sodium and potassium adduct peaks)</td>
<td>Switch off the air condition or use a clean room</td>
</tr>
<tr>
<td>Low S/N ratio and few peaks</td>
<td>Bad quality of AnchorChip target</td>
<td>Insufficient washing of the AnchorChip target</td>
</tr>
<tr>
<td>Low S/N ratio and few peaks</td>
<td>Bad sample quality</td>
<td>False washing procedure</td>
</tr>
<tr>
<td>Low S/N ratio and few peaks</td>
<td>Bad sample quality</td>
<td>AnchorChip target is too old. Do not use the AnchorChip target more than 50 times for the sophisticated ClinProt workflow!</td>
</tr>
<tr>
<td>Low S/N ratio and few peaks</td>
<td>Bad sample quality</td>
<td>Check sample composition in terms of detergents, lipids or other impurities, additives and stabilizers. Use a well-defined control or standard sample to verify the bead-based workflow, e.g. Bruker’s Peptide Calibration Standard I.</td>
</tr>
<tr>
<td>Low S/N ratio and few peaks</td>
<td>Bad sample quality</td>
<td>Check sample storage conditions, e.g. storage at 4°C increases dramatically the proteolysis.</td>
</tr>
<tr>
<td>Problem</td>
<td>Reason</td>
<td>What to do</td>
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<tr>
<td>Suboptimal instrumental</td>
<td>Possibly, accumulate more</td>
<td>Check the instrument settings using Bruker’s ClinProt</td>
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<tr>
<td>settings</td>
<td>single spectra (S/N will be</td>
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<td>improved by the square root</td>
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<td>of numbers of accumulated</td>
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<td>spectra)</td>
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<td>Too much or too few sample</td>
<td>Check the binding conditions</td>
<td>Change the binding</td>
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<td>including sample, magnetic bead</td>
<td>including sample, magnetic bead and binding</td>
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<td>and binding solution volume. Note,</td>
<td>solution volume. Note, that</td>
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<td>that too much sample can lead to</td>
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<td>ion suppression effects.</td>
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<td>Poor reproducibility</td>
<td>Too few accumulations in total</td>
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<td>Adduct peaks</td>
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