5. Process OMX-S® peptides for mass spectrometry identification

The OMX-S protocol yields 20 µl peptides in 45 mM NH_4HCO_3 (**OMX-S®** peptides). We recommend performing *one* of the following protocols before MALDI or ESI based mass spectrometry.

Protocol	Method for sample processing	Mass spectrometry	
A	**Direct use**	**MALDI**	**LC-ESI**
B	**Vacuum concentration**	**MALDI**	**LC-ESI**
C	**Manual RP-microcolumn concentration and desalting**	**MALDI**	**ESI**

A) Direct use of peptide solution

MALDI-MS

OMX-S® delivers the peptide solution in a volume of 20 µl. For application to MALDI target plates, refer to the manufacturers protocol for mixing peptide and matrix solution and loading. *Upon direct use of the OMX-S® peptide solution on standard MALDI targets, note that only part of your peptide volume is used.* For analysis of peptides from low amount of protein, we recommend that you concentrate your complete sample (protocol B), or use a RP-microcolumn (protocol C) or target plates with reverse phase (RP) coating to concentrate and desalt your peptide solution before MALDI analysis.

LC-ESI MS

For automated RP concentration and desalting in LC-ESI MS, the sample may be loaded iteratively according to the sample loop size. The peptide solution should be free of particles.

Step	Detailed instruction
1	Transfer the solution to a reaction tube and centrifuge for 5 min at maximum speed.
2	Withdraw the solution from the top of the liquid surface. Aspirate the liquid slowly. Do not touch the bottom of the reaction tube. Leave a rest of the liquid in the reaction tube to avoid a take up of particles. Transfer the peptide solution to a LC sample vial.

B) Vacuum concentration of sample for MALDI- and LC-ESI MS

Step	Detailed instruction
1	Transfer the peptide solution to a 1.5 ml reaction tube.
2	Put the reaction tube with open lid into the vacuum centrifuge and start the process. A heating of the chamber to 60°C accelerates the drying process and does not decrease peptide recovery.
3	After all of the liquid has evaporated, dissolve the peptides in an appropriate volume of 0.1% FA in water for (LC) ESI-MS[1] ***or*** 0.1% TFA in 50% acetonitrile for MALDI-MS[2]. Incubate for 5 min at room temperature or in an ultrasonic bath.

[1] A particle-free sample transfer is described in protocol A (LC-ESI-MS).
[2] For MALDI-MS desalting is advisable. Please refer to the literature for protocols. For combined concentration and desalting, please use protocol C for RP-microcolumns or target plates with reverse phase (RP) coating!

C) Manual RP-microcolumn concentration and desalting for MALDI- and offline ESI MS

Detailed instruction is provided by the microcolumn manufacturer! A modification of the ZipTip®$_{C18}$ (Millipore, Cat-No. ZTC1 8S0) protocol is described here.

Step	Detailed instruction
1	Activate the ZipTip® by aspirating and dispensing 20 µl of acetonitrile five times. Attention: Avoid running the tip dry. This will result in loss of binding capacity!
2	Remove the acetonitrile from the tip by aspirating and dispensing 20 µl of formic acid (0.1%) five times. Avoid running the tip dry.
3	Bind the peptides to the matrix by aspirating and dispensing the volume of the peptide solution three times. Avoid running the tip dry.
4	Wash the matrix bound peptides by aspirating and dispensing 20 µl of formic acid (0.1%) ten times.
5	Remove all liquid from the tip and detach the tip from the pipette.
6	Elute the peptides with 3 µl of 65% ACN, 1% 2-propanol and 0.1% formic acid for ESI-MS or 0.1% TFA for MALDI-MS, respectively. Proceed as follows: Pipette the solution with a gel loader tip into the ZipTip® on the top of the matrix. Place the ZipTip® into a 0.5 ml reaction tube and attach the ZipTip® back onto the pipette. Press the liquid through the matrix into the reaction tube.
7	Load an appropriate volume into the glass capillary for offline ESI-MS or on the MALDI target.

6. Troubleshooting guide for in-gel digestion

Problem	Step	Possible Cause	Solution
Gel stays pinned in the picker after centrifugation	**5**	Centrifugal force too low	Higher rcf value or longer centrifugation time
Incomplete digestion	**1**	Incorrect pH-value because of insufficient gel washing	Wash the gel intensely with water before cutting the spot
	7	Incorrect pH-value of reagents	Ensure that pH of buffer is ~ 8.0
	7	Low enzyme activity	Use a new Trypsin aliquot
Low volume yield of peptide solution after post-digestion centrifugation	**10**	Centrifugal force too low	Increase rcf value or centrifugation time
Gel particles in the peptide solution after post-digestion centrifugation	**10**	Centrifugal force to high	Set rcf value and centrifugation time as recommended in the protocol