Proteome X Solutions

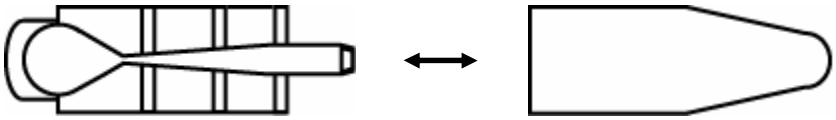
OMX-S® for rapid in-gel digestion

OMX-S® *basic* Instruction Manual April/08

Part No.: 17000

Table of Contents

The OMX-S® device



Reactor enabling the in-gel digestion of proteins

Picker to cut protein from bands or spots of electrophoresis gel

Peptide-Sampler to collect the peptide solution after the digestion process

1. Introduction

This guide features a detailed step-by-step protocol for the **OMX-S®** device (p. 3-5). **OMX-S®** is a three-in-one tool featuring a combination of

- a ***Picker*** for cutting protein bands or spots from an electrophoresis gel
- a ***Reactor*** enabling the tryptic in-gel digestion of protein spots
- a ***Sampler*** collecting the peptide solutions from the reactor

The guide also gives recommendations for reduction and alkylation of proteins before electrophoresis (p. 2), and for the processing of peptides before identification by mass spectrometry (p. 6-7).

With **OMX-S®**, you will accomplish a rapid, robust, and reproducible in-gel digestion of proteins. You will pick proteins from 1D and 2D electrophoresis gels and perform a contamination-free peptide sample preparation. The cost effective OMX technology provides highest peptide yield for high quality mass spectra in MALDI- and ESI-MS.

2. Precautions and important notices

OMX-S® is developed for laboratory use only. No hazardous components are contained within **OMX-S®**. Nevertheless, appropriate safety apparel such as lab coat, eye protection and especially cleaned gloves should be worn. These precautions help to avoid sample contamination especially with traces of keratin.

OMX-S® is manufactured for single use. Until use, **OMX-S®** should remain stored in the original package under clean conditions at room temperature in order to avoid any contamination. The device should be used within 12 months from the date of purchase. The reactor of **OMX-S®** contains flexible components. We therefore guarantee proper efficiency only if the reactor remains closed.

The average weight of an empty **OMX-S®** is 1.46 g. Please, use a suitable tare, if only one **OMX-S®** is used in a centrifuge.

3. Reduction and alkylation of proteins

For homogeneous cysteine derivatization, we recommend reduction and alkylation of proteins before SDS-PAGE.

#	Description	
1		Solubilize your proteins in a standard sample buffer (e.g. Laemmli sample-buffer [Ref. 1]).
2	15 min/60°C	Add DTT to a final concentration of 10 mM. Incubate in a thermo block.
3		Cool the solution to room temperature.
4	15 min/RT	Add iodoacetamide to a final concentration of 60 mM. Incubate in the dark.
5		Load the samples on the SDS-gel

4. Protocol for tryptic in-gel digestion using OMX-S®

Use **OMX-S®** for tryptic in-gel digestion of protein bands or spots from 1D and 2D-SDS-PAGE-gels which are stained with soluble or colloidal Coomassie® G250 blue dye.

A) Chemicals and lab equipment to be supplied by the user

Chemicals

- *Water (Molecular Biology Grade, 18 MΩ or equivalent)*
- *Digestion buffer;*
  NH_4HCO_3 (50 mM, pH ca. 8.0)
- *Trypsin stock solution:*
  Dissolve the 20 µg of modified trypsin (Promega/2005/Cat.-Nr.: V5111) according to the manufacturers protocol in 200 µl of the resuspension buffer (50 mM acetic acid) supplied by the manufacturer of the enzyme.
- *Trypsin working solution:*
  Prepare a 1/10 dilution of *Trypsin stock solution* in *Digestion buffer.*

Lab equipment

- *A clean, flat tray for washing the gel*
  The tray size depends on the gel volume. Per cm^3 gel, a volume of at least 20 ml water is recommended.
- *A clean glass plate*
- *A light table (optional)*
- *A clean bench top micro centrifuge suitable for 1.5 ml standard tubes*
  The centrifugation parameters are in units of relative centrifugal force (rcf).
- *Pipettes (designed for pipetting volumes of 18 µl and 2 µl, respectively); e.g. Gilson (10 and 20 µl), Eppendorf (10 µl)*
- *Thermomixer suitable for 1.5 ml standard tubes*
  A temperature of 50°C and a mixing-speed of 1000 rpm are recommended.

B) Step by step protocol for tryptic in-gel digestion using OMX-S®

#	Description		
1	2 x 5 min	Wash gel slab in water.	
2		Drip off excessive water and transfer the gel to a cleaned glass plate.	
3		Detach the Peptide-Sampler from an **OMX-S®** and put it on the bottom side of the Reactor.	Fig. 1/1
4		Excise protein band or spot with the Picker. Picking can be repeated up to two times. After picking, lift picker as shown.	Fig. 2
5	**2 min 13.000 x g**	Close the **OMX-S®** by screwing the Sampler back on the picking side of the Reactor. Do not over tighten. Place the **OMX-S®** in the centrifuge with the **Reactor** placed **at the bottom** of the centrifuge. Spin down gel	Fig. 1/2
6		Remove the **OMX-S®** from the centrifuge and detach the Peptide-Sampler. Do not attach the Sampler on the bottom side of the Reactor! Pipette 20 µl* of *Trypsin working solution* into the Picker. Close the **OMX-S®** by screwing the Sampler back on the picking side of the Reactor.	
7	**Short spin 3800 x g**	Place the **OMX-S®** in the centrifuge. Ensure that the **Reactor** is placed **at the bottom** of the centrifuge. Spin down the liquid.	Fig. 1/2
8	45 min/50°C [Ref. 2-4]	Place the **OMX-S®** in a thermomixer with the **Reactor** positioned **at the bottom** of the thermo unit. Incubate with agitation (500 rpm).	Fig. 1/3
9	**3 min 1.000 x g**	Place the **OMX-S®** in the centrifuge. Ensure that the **Peptide-Sampler** is placed **at the bottom** of the centrifuge. Spin down peptide solution.	Fig. 1/4
10		For short time (≤ 24h) the peptide solution can be stored at +4°C. If longer times of preservation are intended, store the peptide solution at –20°C.	
11		Transfer peptides to MS. Continue on page 6	

*Volumes of 20 µl are inserted into the Picker with pipettes equipped with standard 200 µl tips. In case of difficulties, we recommend to pipette the liquid directly into the Reactor. In this case, use tips with a thinner tip end e.g. from Brand (pipette tips, 2005/Cat.-Nr. 702504) or from Eppendorf (gel loader tips, 2005/Cat.-Nr. 0030 001.222). Maximal loading capacity of the Reactor is 40 µl.

Fig. 1 Flow Chart

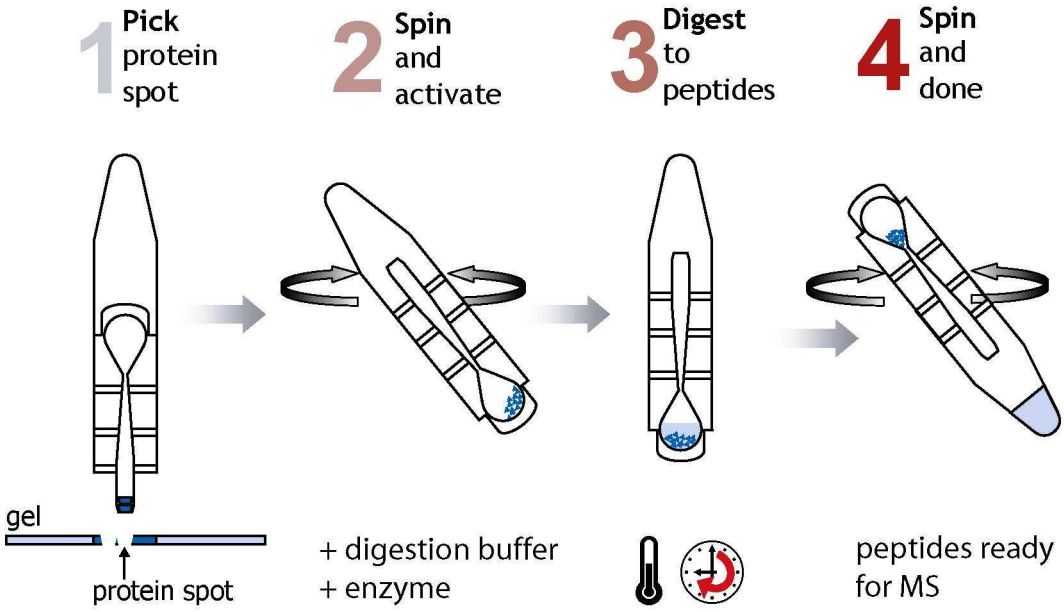
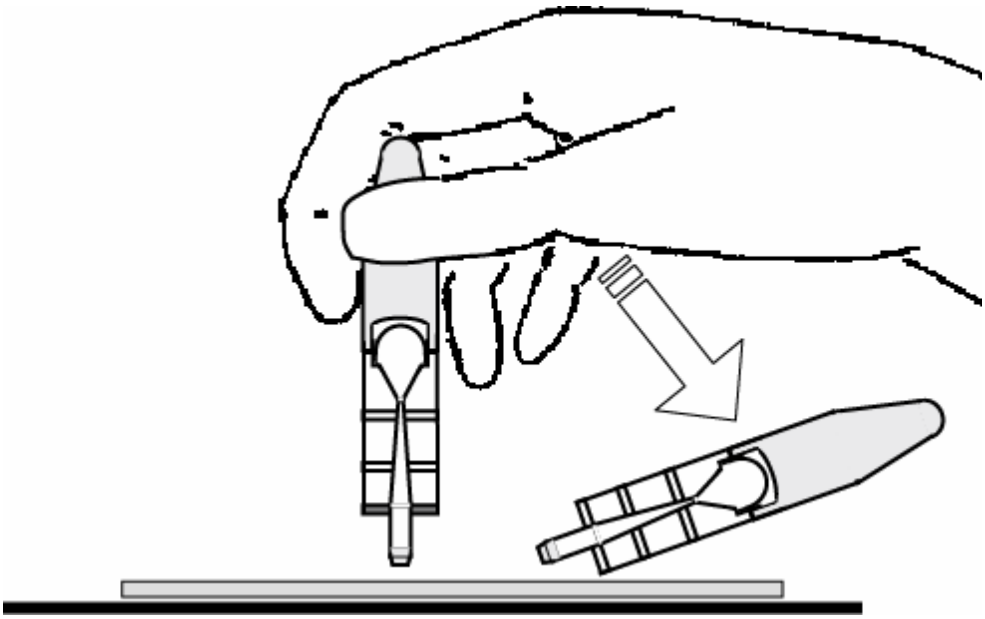


Fig. 2 Illustration of the picking process



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

7. Technical data sheet

OMX-S® length:	50 mm
Rotor cavity diameter required:	11 mm
Maximum Reactor volume:	40 µl
Maximum spin speed:	13000 x g
Diameter of picker:	1.8 mm
Materials:	Polypropylen and glass
Storage:	**OMX-S®** should be stored dry and clean at room temperature and protected from UV-light. **OMX-S®** is stable for at least 1 year when stored as described.
Resistance to chemicals:	Resistant to water, diluted acids, short chain alcohols, and acetonitrile. Not resistant to hydrocarbons, arenes, and halogenated hydrocarbons.

8. Ordering information

Manufacturer:
OMX GmbH
Information and Technical assistance: info@omx-online.com

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D-82234 Wessling
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The product is delivered with documentation including specifications and technical information. **OMX-S®** is only for use referring to supplied instruction manual. The information in this manual is provided in all conscience. All specifications are subject to change without prior notice. Conditions of product application are uncontrollable by the manufacturer. Therefore, we do not warrant the suitability of the product for particular use or positive results. The customer or user agrees to all risks and liabilities. OMX GmbH shall not be liable for any loss or direct, incidental, consequential or special damage caused from the use or misuse of the product.

References:

1) Laemmli, U. K., Nature, 227, 680–685 (1970)
2) Havlis, J. *et al.*, Anal Chem., 75(6), 1300-1306 (2003).
3) Finehout, E. J. *et al.*, Proteomics, 5(9), 2319-2321 (2005).
4) Finehout E. J, *et al.*, Electrophoresis, 24(19-20), 3508-16 (2003)