Proteome X Solutions

OMX-S® for rapid in-gel digestion

OMX-S® *pro* Instruction Manual*
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* Please, check for latest version at www.omx-online.com.

1. Introduction

With OMX-S® *pro*, existing protocols for the in-gel digestion of protein bands can be accomplished in one single device. Sample preparation with OMX-S® *pro* is recommended for all samples where additional processing steps like destaining and reduction & alkylation are intended. OMX-S® *pro* enables you to accomplish a significantly simplified and contamination-free sample preparation. The OMX technology is cost effective, provides high peptide yields and better quality mass spectra.

The **OMX-S®** device 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 in-gel digestion of the protein spots
- a ***Sampler*** collecting solutions from the reactor

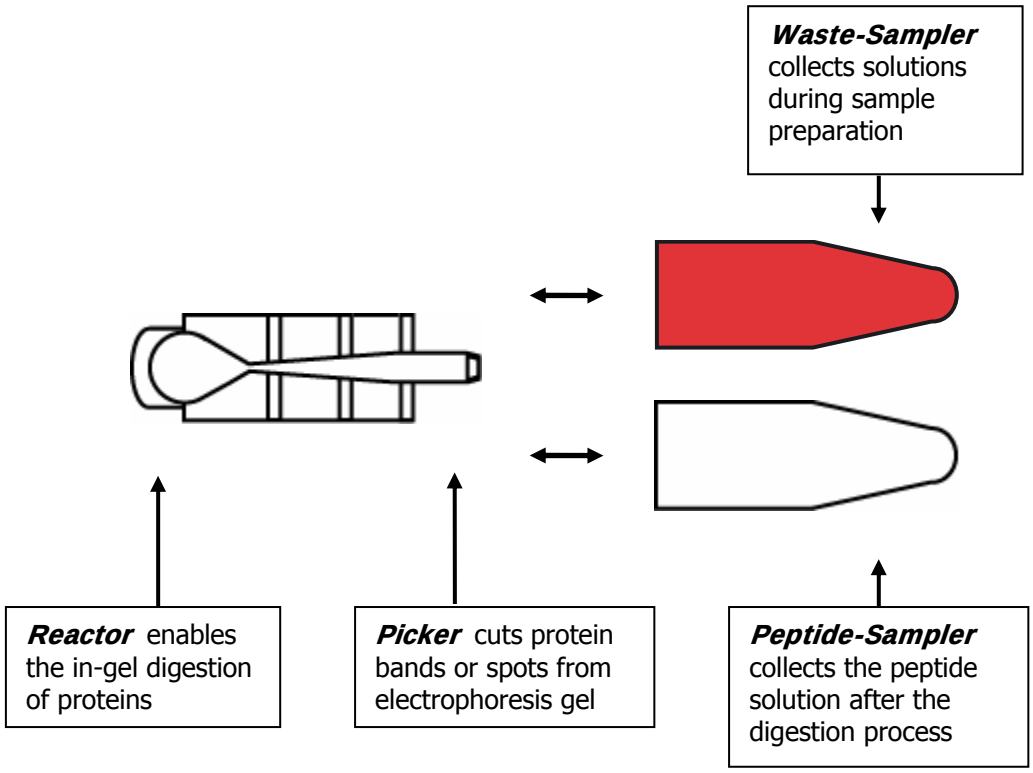
For collection of the peptide solution after the digestion process, a separate Peptide-Sampler is provided. This guide features a detailed step-by-step protocol for the tryptic in-gel digestion of proteins picked from electrophoresis gels enabling a rapid and reproducible sample preparation.

2. Content and precautions

2.1. Content

This package contains 12 OMX-S® with attached Waste-Samplers, 12 separate Peptide-Samplers, 30 pipette tips, and the instruction guide.

2.2. Description of the OMX-S® device



The OMX-S® device is delivered with Waste-Sampler attached on the Picker-bearing side of the Reactor. The Waste-Sampler is intended for all sample preparation steps except for the final collection of the peptide solution. For collection of the peptide solution, the Waste-Sampler should be replaced by the Peptide-Sampler to exclude any contamination from preceding steps.

2.3. 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.
The OMX-S® device 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. Protocols

3.1. Detailed protocol for rapid tryptic in-gel digestion in OMX-S®

To efficiently use OMX-S® for tryptic in-gel digestion we provide a step-by-step protocol for the analysis of protein bands or spots from 1D and 2D-SDS-PAGE-gels. It is recommended to stain proteins with soluble or colloidal Coomassie® blue dye. For efficient digestion and high peptide recovery, it is essential not to fix stained proteins within the gel matrix using e.g. formaldehyde or glutaraldehyde. After operation of the OMX® protocol, it is recommended to desalt and concentrate the resulting peptide solution on micro RP-silica tips. In case of direct use for LC-MS, please ensure that the solution contains no micro gel particles. The following chemicals and laboratory equipment are recommended, but are not supplied:

Chemicals

- *Water (Molecular Biology Grade, 18 MΩ or equivalent)*
- *Digestion buffer:*
  Tris buffer (50 mM, pH 8.0) ***or*** *ammonium bicarbonate solution (50 mM, pH ca. 8.0)*
- *Trypsin stock solution:*
  For tryptic in-gel digestion, 20 µg of **modified** trypsin (Promega/2005/Cat.-Nr.: V5111) is dissolved according to the manufacturers protocol in 200 µl resuspension buffer (50 mM acetic acid which is supplied by the manufacturer of the enzyme). 2 µl of this stock solution (= 200 ng trypsin) are used per sample.
- *Working solution:*
  The working solution has to be prepared fresh before use. For **one** sample, 2 µl of trypsin stock solution are added to 18 µl of digestion buffer. If more than one sample is prepared at the same time, volumes are multiplied with the number of samples (e.g. working solution for 10 samples: 20 µl of trypsin solution + 180 µl of 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.

Detailed tryptic digestion protocol

#	Description	Detailed instruction	
1	Preparation of the gel	Wash gel slab in water.	2 x 5 min at RT
2		Drip off excessive water and transfer the gel to a cleaned glass plate.	
3	Excision of spots	Take an OMX-S®, detach the Waste-Sampler from the Reactor, and put the Waste-Sampler on the bottom side of the Reactor (Fig. 2/1, p. 5).	
4		Excise the protein band or spot of interest with the Picker (Fig. 1, p. 5). After picking, lift picker as shown in Fig. 1 (p. 5).	
4a		Repeat step 4 up to two times if excision of bigger spots is intended.	
5	Crush the gel	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. 2/2 p. 5)	**2 min at rcf 13.000 x g**
6		Remove the OMX-S® from the centrifuge and detach the Waste-Sampler. Attach it on the bottom side of the Reactor and place the device in a rack.	
(A)	*optional Destaining*	*ref. to C1 –C5/p. 6: Coomassie stained plugs* *ref. to S1- S7/p. 7: Silver stained plugs*	
(B)	*optional Reduction & alkylation*	*ref. to R1 – R10/p. 8: Reduction & alkylation*	
7		Remove cap from the Peptide-Sampler and **replace the Waste-Sampler by the Peptide-Sampler.**	
8	Digestion of proteins	Pipette 20 µl of working solution (refer to p. 3) into the Picker. *	
9		Place the OMX-S® in the centrifuge. Ensure that the **Reactor** is placed **at the bottom** of the centrifuge. Spin down the liquid. (Fig. 2/2 p. 5)	**Short spin at rcf 3800 x g**
10		Place the OMX-S® in a thermomixer with the **Reactor** positioned **at the bottom** of the thermo unit and incubate under agitation (1000 rpm) (Fig. 2/3 p. 5).	45 min at 50°C [Ref. 1-3]
11	Separation of gel and peptide solution	Place the OMX-S® in the centrifuge. Ensure that the **Peptide-Sampler** is placed **at the bottom** of the centrifuge. Spin down the peptide solution (Fig. 2/4 p. 5).	**3 min at rcf 1.000 x g**

*The enclosed Brand pipette tips (2005/Cat.-Nr. 702504) fit onto 10 µl and 20 µl pipettes from Gilson and 10 µl pipettes from Eppendorf. If other pipettes are used, you could cut the end of a standard pipette tip and use this as an adapter to plug into the enclosed pipette tip. If addition of higher volumes is intended, please provide liquid stepwise and centrifuge between steps or use gel loader tips e.g. Eppendorf (2005/Cat.-Nr. 0030 001.222) to pipette the liquid directly into the Reactor. Maximal loading capacity of the Reactor is 40 µl.

Fig. 1 Illustration of the picking process:

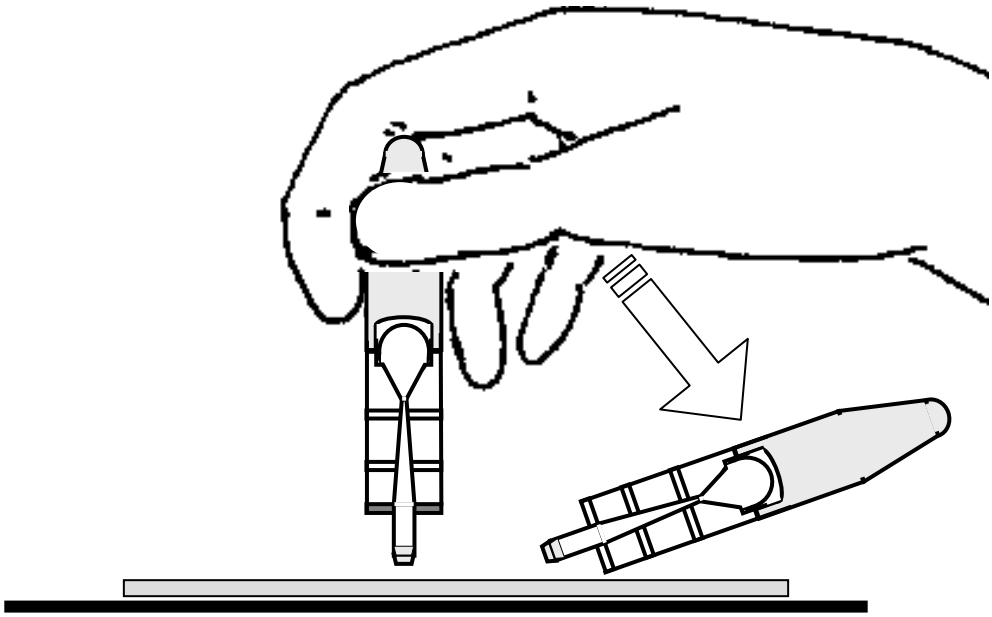
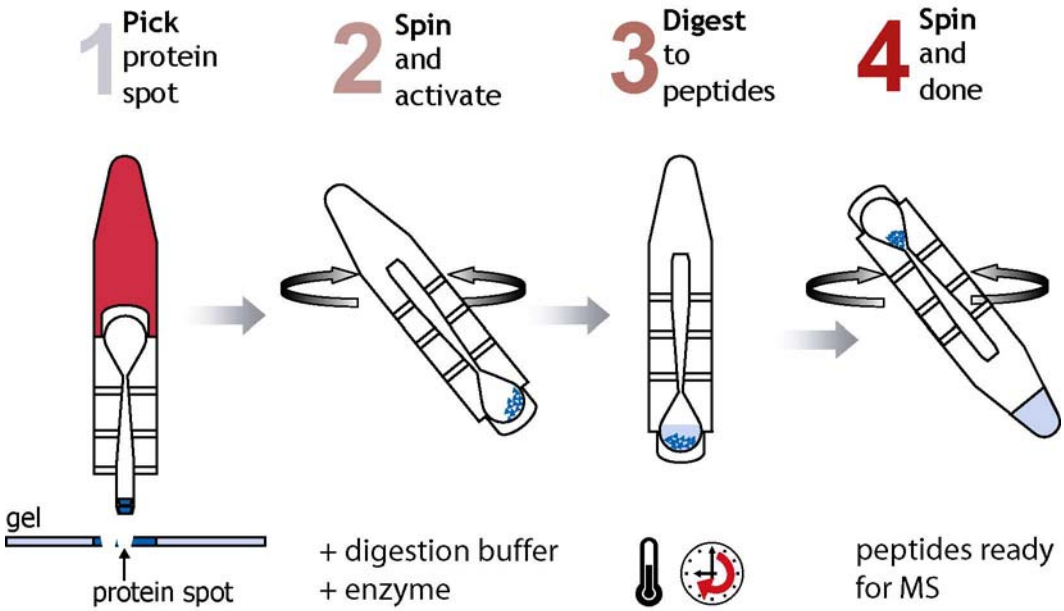


Fig. 2 Flow Chart:



3.2. Coomassie Blue destaining in OMX-S®

Destaining of Coomassie® Brilliant Blue (CBB) stained spots is not obligatory before in-gel digestion, because CBB does not interfere with the enzymatic cleavage. In the mass spectra, CBB R250 molecules form two single charged peaks in positive ion mode spectra, which can be detected at 804 m/z [$+H^+$] and 826 m/z [$+Na^+$]. Peptide signal detection may only be affected in cause of overlapping peptide and CBB signals.

Destaining of the protein in the OMX-S® device is efficient and needs only 10-15 minutes. Please refer to the following instruction:

Chemicals

- *Acetonitrile*
- *Digestion buffer:*
  Tris buffer (50 mM, pH 8.0) ***or*** *ammonium bicarbonate solution (50 mM, pH ca. 8.0)*

C1	**Coomassie destaining**	Pipette first 7.5 µl digestion buffer and afterwards 7.5 µl acetonitrile or 15 µl of a 1:1 mixture in the Picker.	
C2		Put the Waste-Sampler back on the picking side of the Reactor. Place the OMX-S® in the centrifuge with the **Reactor at the bottom** of the centrifuge. Spin the destaining solution into the reaction chamber.	**Short spin at rcf 3800 x g**
C3		Place the OMX-S® in a thermomixer with the **Reactor** positioned **at the bottom** of the thermo unit, and incubate under gentle agitation	10 min at 37°C
C4		Place the OMX-S® in the centrifuge. Ensure that the **Waste-Sampler** is placed **at the bottom** of the centrifuge. Spin down and **remove** the destaining solution	**3 min at 1000 x g**
C5		To continue with the digestion procedure, refer to tryptic digestion protocol at step 7, page 4. For reduction and alkylation continue with step R1, page 8.	

3.3. Silver destaining [Ref. 4] in OMX-S®

Destaining of the protein in the OMX-S® device is efficient and needs only 15 minutes. Please refer to the following instruction:

Chemicals

- *Solution 1: 6 mM potassium hexacyanoferrate(III) ($K_3[Fe(CN)_6]$) in water*
- *Solution 2: 20 mM sodium thiosulfate ($Na_2S_2O_3$) in water*
- *Digestion buffer:*
  Tris buffer (50 mM, pH 8.0) ***or*** *ammonium bicarbonate solution (50 mM, pH ca. 8.0)*

S1	**Silver destaining**	Pipette 10 µl of solution 1 and 10 µl of solution 2 into the Picker	
S2		Put the Waste-Sampler back on the picking side of the Reactor. Place the OMX-S® in the centrifuge with the **Reactor at the bottom** of the centrifuge. Spin the destaining solution into the reaction chamber.	**5 min at 3800 x g**
S3		Turn the OMX-S® around, spin down and **remove** the destaining solution.	**3 min at 1000 x g**
S4	Washing of the gel	Pipette 20 µl of digestion buffer (without enzyme) into the Picker.	
S5		Put the Waste-Sampler back on the picking side of the Reactor. Place the OMX-S® in the centrifuge with the **Reactor at the bottom** of the centrifuge. Spin the washing solution into the reaction chamber.	**5 min at 3800 x g**
S6		Turn the OMX-S® around, spin down and **remove** the washing solution.	**3 min at 1000 x g**
S7		To continue with the digestion procedure, refer to tryptic digestion protocol at step 7, page 4. For reduction and alkylation continue with step R1, page 8.	

3.4. Reduction & alkylation in OMX-S®

If reduction & alkylation is essential, it is advisable to perform this procedure prior to electrophoresis in order to prevent the formation of cysteine-acrylamide adducts [5, 6]. In cases when reduction & alkylation shall be accomplished between electrophoresis and digestion, it can be easily implemented in the OMX-S® device according to the following procedure. Please refer to the following instruction:

Chemicals

- *dithiothreitol (DTT)*
- *iodoacetamide (IAA)*
- *ammonium bicarbonate*

Solutions

- *ammonium bicarbonate solution (50 mM, pH ca. 8.0)*
- *DTT solution: 10 mM in ammonium bicarbonate solution (corresponds to about 1,5 mg DTT/ml)*
- *IAA solution: 55 mM in ammonium bicarbonate solution (corresponds to about 10 mg iodoacetamide/ml)*

R1	**Reduction & alkylation**	Pipette 20 µl of DTT solution into the Picker	
R2		Put the Waste-Sampler back on the picking side of the Reactor. Place the OMX-S® in the centrifuge with the **Reactor at the bottom** of the centrifuge. Spin the DTT solution into the reaction chamber.	**Short spin at rcf 3800 x g**
R3	**Reduction**	Place the OMX-S® in a thermomixer with the **Reactor** positioned at the bottom of the thermo unit, and incubate under gentle agitation.	15 min at 50°C
R4		Place the OMX-S® in the centrifuge. Ensure that the **Waste-Sampler** is placed **at the bottom** of the centrifuge. Spin down and **remove** the DTT solution.	**3 min at 1000 x g**
R5		Pipette 20 µl of IAA solution into the Picker.	
R6		Put the Waste-Sampler back on the picking side of the Reactor. Place the OMX-S® in the centrifuge with the **Reactor at the bottom** of the centrifuge. Spin the IAA solution into the reaction chamber.	**Short spin at rcf 3800 x g**
R7	**Alkylation**	Put the OMX-S® in a dark place (e.g. in the centrifuge) with the **Reactor** positioned **at the bottom** and incubate.	15 min at room temperature
R8		Place the OMX-S® in the centrifuge. Ensure that the **Waste-Sampler** is placed **at the bottom** of the centrifuge. Spin down and **remove** the IAA solution.	**3 min at 1000 x g**
R9		To continue with the digestion procedure, refer to tryptic digestion protocol at step 7, page 4.	

4. Troubleshooting

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	11	Centrifugal force too low	Increase rcf value or centrifugation time
Gel particles in the peptide solution after post-digestion centrifugation	11	Centrifugal force to high	Set rcf value and centrifugation time as recommended in the protocol
CBB destaining incomplete	C3	Intensively stained spot	Increase incubation time
Silver destaining incomplete	S2	Intensively stained spot	Increase incubation time

5. 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.

6. Ordering information

Manufacturer:
OMX GmbH

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D-82234 Wessling
Germany

Information and Technical assistance: www.omx.de

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Status:18.04.2008

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:

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