Proteome X Solutions

OMX-S® for rapid in-gel digestion

OMX-S® *pro* Kit Instruction Manual* Nov/08

Part No.: 17003

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* Please, check for latest version at www.omx-online.com.

1) Introduction

With OMX-S®, existing protocols for the in-gel digestion of protein bands can be accomplished in one single device. Sample preparation is rapid, significantly simplified and the risk of contamination is minimized. The OMX technology is cost effective, provides high peptide yields and better quality mass spectra.

The Kit contains OMX-S® devices as well as additional tools and chemicals for protein sample preparation. The Kit is intended for protein in-gel digestion in the OMX-S® device and according to your requirements also for the procedures of Coomassie™ destaining of gel plugs, silver destaining of gel plugs, as well as the reduction and alkylation of gel-bound proteins.

This manual features a detailed step-by-step protocol for the tryptic in-gel digestion of proteins from electrophoresis gels enabling a rapid and reproducible sample preparation. Furthermore, this manual details step-by-step protocols for additional sample preparation procedures like Coomassie™ destaining, silver destaining and reduction & alkylation of gel-bound proteins.

2) Content

2.1) Kit components

Description	Volume*
12 OMX-S® devices	
12 Peptide Sampler	
30 Pipette tips	
Instruction guide	
Modified Trypsin (4 µg, red cap)	40 µl
Acetic acid (50 mM, green cap)	200 µl
Digestion buffer (pink cap)	10 ml
Silver destaining solution 1 (yellow cap)	1.0 ml
Silver destaining solution 2 (colourless cap)	1.0 ml
Reduction reagent (blue cap)	1.0 ml
Alkylation reagent (white cap)	3x1.0 ml

*Reagents and enzyme except acetic acid are supplied as dry substance. The amount of reagents is sufficient for at least 12 reactions.

2.2) Storage conditions

The OMX-S® device is manufactured for single use. Store the OMX-S® devices, the pipette tips and the waste sampler in the original package at room temperature under dry and clean conditions and protected from UV-light. The reactor of OMX-S® contains flexible components. We therefore guarantee proper efficiency only if the reactor remains closed.

OMX-S® devices are stable for at least 1 year when stored as described.

After unpacking the plastic items, the chemicals of the OMX in-gel digestion kit should be stored in the original box at 2-8°C. Dissolved chemicals (except alkylation reagent!) of the OMX in-gel digestion Kit can be stored at - 20 °C. Trypsin has to be stored at -20 °C. Reconstituted trypsin is stable for at least 3 months.

2.3) Additionally required chemicals and equipment

The following chemicals and lab equipment are required but not supplied in the Kit:

Chemicals and Enzymes:

- *Water*: Molecular Biology Grade, 18 megaohm or equivalent
- *Acetonitrile (optional)*: for Coomassie™ destaining

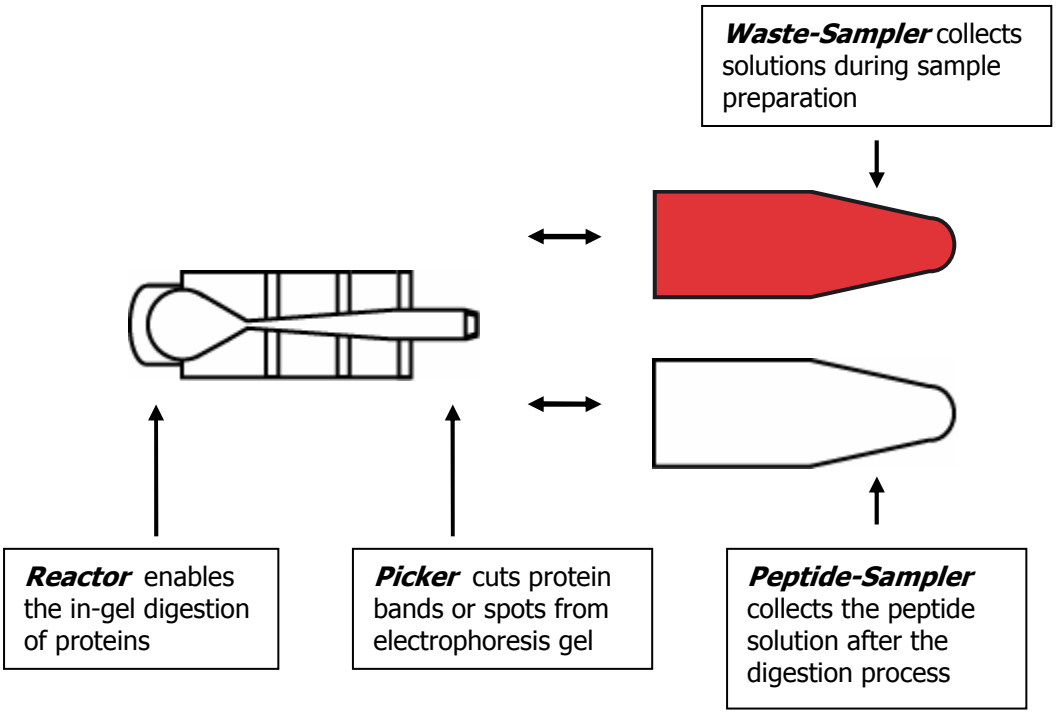
Lab equipment:

- *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.

2.4) 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 particularly with traces of keratin.

2.5) Description of the OMX-S® device



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 and a ***Sampler*** collecting solutions from the reactor. The 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 after the digestion process, a separate Peptide-Sampler is provided. The average weight of an empty OMX-S® is 1.46 g. 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.

3.2) Prearrangements

Preparation of buffers and solutions supplied with the Kit:

Digestion buffer (pink cap)
Add **10 ml of water** and mix briefly to dissolve the white powder. The solution is now ready for use. The solution should be stored at -20 ºC if not in use.

Trypsin stock solution (red cap)
The supplied trypsin (4 µg) is dissolved in 40 µl acetic acid (50 mM, green cap). 2 µl of this stock solution (= 200 ng trypsin) are used per sample. The solution should be stored at -20 ºC if not in use.

Silver destaining solution 1 (yellow cap)
Add **1.0 ml of water** and mix briefly to dissolve the orange powder. The solution is now ready for use. The solution should be stored at -20 ºC if not in use.

Silver destaining solution 2 (colourless cap)
Add **1.0 ml of water** and dissolve the white powder. The solution is now ready for use. The solution should be stored at -20 ºC if not in use.

Reduction reagent (blue cap)
Add **1.0 ml of digestion buffer** and dissolve the white powder. The solution is now ready for use. The solution should be stored at -20 ºC if not in use.

Alkylation reagent (white cap)
Add **1.0 ml of digestion buffer** to a vial and mix briefly to dissolve the white powder directly before use. A freshly prepared solution is required for alkylation. The solution cannot be stored.

Preparation of additional buffers and solutions:

Working solution:
The working solution has to be prepared fresh before use. For **one** sample, **2 µl of trypsin stock solution are mixed with 18 µl of digestion buffer (pink cap) in a separate reaction tube** (not supplied). 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).

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. 7).	
4		Excise the protein band or spot of interest with the Picker (Fig. 1, p. 7). After picking, lift picker as shown in Fig. 1 (p. 7).	
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. 7)	**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. 8: Coomassie stained plugs* *ref. to S1- S7/p. 9: Silver stained plugs*	
(B)	*optional Reduction & alkylation*	*ref. to R1 – R10/p. 10: 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. 5) 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. 7)	**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. 7).	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. 7).	**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:

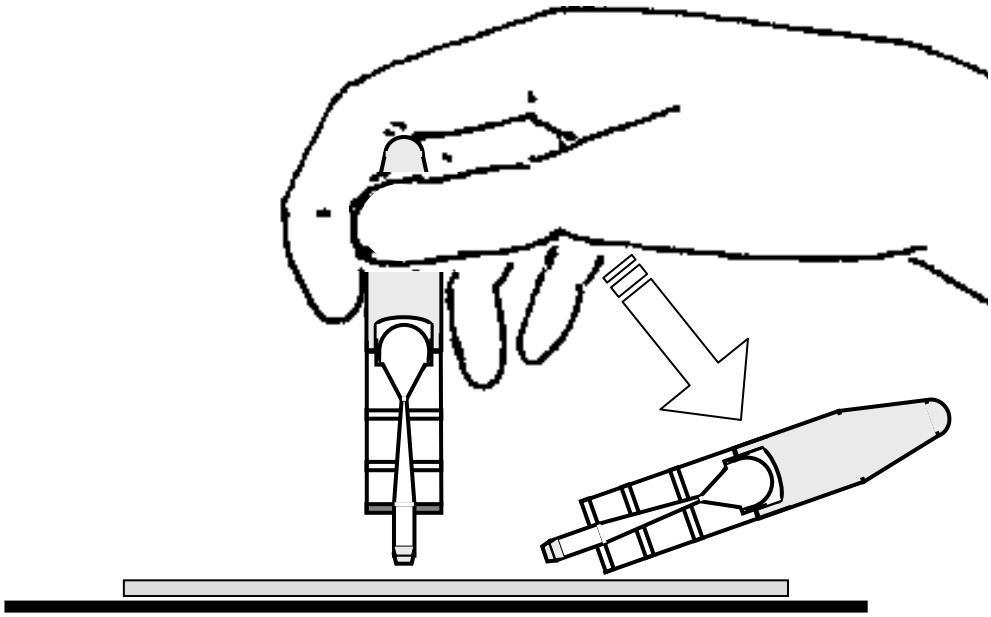
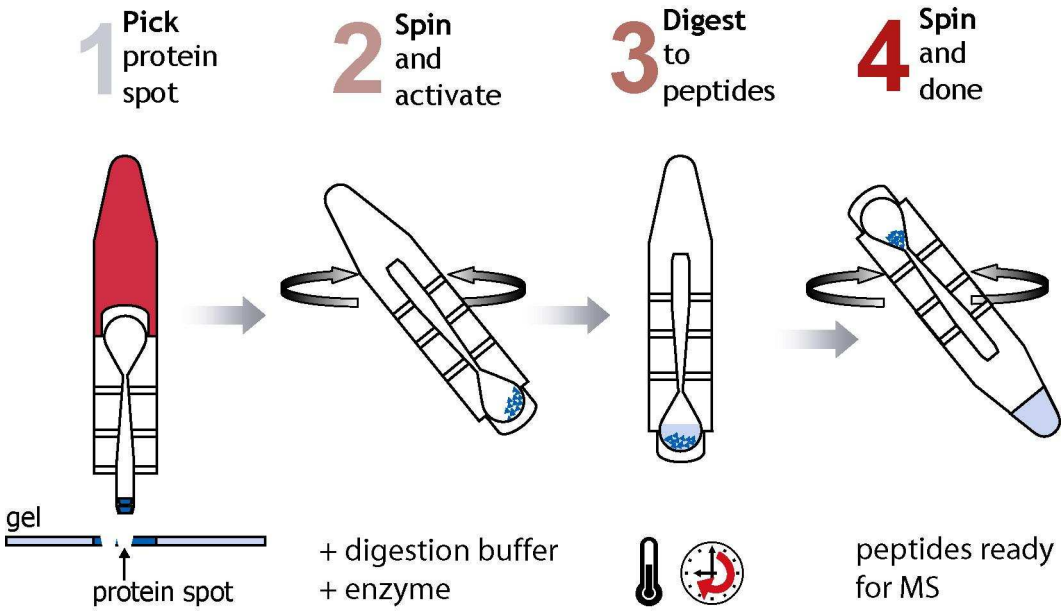


Fig. 2 Flow Chart:



3.3) Coomassie Blue destaining in OMX-S®

Destaining of CoomassieTM Brilliant Blue (CBB) stained spots is not obligatory before in-gel digestion, because CBB does not interfere with the enzymatic cleavage. CBB molecules are detectable in positive ion spectra. The peak intensity of CBB varies subject to the protein concentration of a gel spot. The signals can be used for internal calibration. 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 (not supplied)
- Digestion buffer (pink cap)

C1	**Coomassie destaining**	Pipette first 7.5 µl digestion buffer (pink cap) 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 6. For reduction and alkylation continue with step R1, page 10.	

3.4) 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 (yellow cap)
- Solution 2 (colourless cap)
- Digestion buffer (pink cap)

S1	**Silver destaining**	Pipette 10 µl of solution 1 (yellow cap) and 10 µl of solution 2 (colourless cap) 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 (pink cap) 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 6. For reduction and alkylation continue with step R1, page 10.	

3.5) 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 [Ref. 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:

- Digestion buffer (pink cap)
- Reduction reagent (blue cap) in digestion buffer
- Alkylation reagent (white cap) in digestion buffer

R1	**Reduction & alkylation**	Pipette 20 µl of reduction solution (blue cap) 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 reduction 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 reduction solution.	**3 min at 1000 x g**
R5		Pipette 20 µl of alkylation solution (white cap) 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 alkylation solution into the reaction chamber.	**Short spin at rcf 3800 x g**
R7	**Alkylation**	Incubate the OMX-S® in a dark place (e.g. in the centrifuge) with the **Reactor** positioned **at the bottom**.	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 alkylation solution.	**3 min at 1000 x g**
R9		To continue with the digestion procedure, refer to tryptic digestion protocol at step 7, page 6.	

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
Average weight	1.46 g
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
Information and Technical assistance: www.omx-online.com

Adelbergweg 14 Tel.: +49-(0)8153-908785-3
D-82234 Wessling Fax: +49-(0)8153-908785-4
Germany

Status: 19.11.2008

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.

7) References

1) Havlis, J. *et al.*, Anal Chem., 75(6), 1300-1306 (2003).
2) Finehout, E. J. *et al.*, Proteomics, 5(9), 2319-2321 (2005).
3) Finehout E. J, *et al.*, Electrophoresis, 24(19-20), 3508-16 (2003)
4) Gharahdaghi F. *et al.*, Electrophoresis, 20(3), 601-5 (1999)
5) Speicher K.D. *et al.*, J. Biomol. Tech., 11, 74-86 (2000)
6) Herbert B. *et al.*, Electrophoresis, 22(10), 2046-2057 (2001)

8) Precautions and important notices concerning the chemicals

Digestion buffer (pink cap)

Composition:	ammonium bicarbonate
CAS No.:	1066-33-7 ammonium hydrogencarbonate
Hazard description:	Xn Harmful R-22: Harmful if swallowed.

Silver destaining solution 1 (yellow cap)

Composition:	potassium ferrycyanide (III)
CAS No.:	13746-66-2 tripotassium hexacyanoferrate
Hazard description:	No hazardous product as specified in directive 67/548/EEC

Silver destaining solution 2 (colourless cap)

Composition:	sodium thiosulfate pentahydrate
CAS No.:	10102-17-7 sodium thiosulfate pentahydrate
Hazard description:	No hazardous product as specified in directive 67/548/EEC

Reduction reagent (blue cap)

Composition:	1,4-dithiothreitol
CAS No.:	3483-12-3 (R*,R*)-1,4-dimercaptobutane-2,3-diol
Hazard description:	Xn Harmful R-22-36/38: Harmful if swallowed. Irritating to eyes and skin.

Alkylation reagent (white cap)

Composition:	2-iodoacetamide
CAS No.:	144-48-9
Hazard description:	Xi Irritant R-36/37/38-43: Irritating to eyes, respiratory system, and skin. May cause sensitization by skin contact.

Trypsin (red cap)

Composition:	modified trypsin from porcine pancreas, NB sequencing grade
CAS No.:	9002-07-7
Hazard description:	Xn Harmful R 36/37/38-42/43 Irritating to eyes, respiratory system and skin. May cause sensitisation by inhalation and skin contact.

Acetic acid (green cap)

Composition:	acetic acid, 50 mM
CAS No.:	64-19-7
Hazard description:	No hazardous product as specified in directive 67/548/EEC