

For life science research only.
Not for use in diagnostic procedures.



Trypsin recombinant, Proteomics Grade recombinant porcine trypsin from *Pichia pastoris*

 **Version: 05**

Content Version: November 2020

Lyophilized, stabilized

Cat. No. 03 708 985 001 4 x 25 µg
Cat. No. 03 708 969 001 4 x 100 µg

Store the product at –15 to –25°C.

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1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Catalog number	Content
1	Trypsin recombinant, Proteomics Grade	Highly purified and specific protease.	03 708 985 001	4 vials, 25 µg each
			03 708 969 001	4 vials, 100 µg each

1.2. Storage and Stability

Storage Conditions (Product)

When stored at –15 to –25°C, the product is stable through the expiry date printed on the label.

Vial / Bottle	Label	Storage
1	Trypsin recombinant, Proteomics Grade	Store at –15 to –25°C.

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- 1 to 10 µl variable pipette
- 1 to 100 µl variable pipette
- 100 to 1,000 µl variable pipette
- Pipette tips
- Desalting/concentrating pipette tips, such as C18 resin ZipTip_{scx} pipette tips, Millipore.
- Microplate, such as v-shape PP-microplate, Greiner
- pH meter
- Measuring cylinder, 100 ml
- Laboratory centrifuge
- Thermomixer
- Vacuum concentrator
- Scalpel

For digestion of proteins in gels

⚠ All reagents must be analytical grade or higher quality.

- Ammonium hydrogen carbonate (NH₄HCO₃), MW 79.06
- Dithiothreitol* (DTT), MW 154.3
- Water, ultrapure
- Iodoacetamide, MW 185.0
- Tris-(hydroxymethyl) aminomethane Tris, MW 121.14
- Trifluoroacetic acid (TFA), MW 114.0
- Acetone, MW 58.08
- Acetonitrile, MW 41.05
- 2 M NH₄OH
- 1 M HCl
- Tris-HCl*

1. General Information

For digestion of proteins in solution

⚠ **All reagents must be analytical grade or higher quality.**

- Dithiothreitol* (DTT), MW 154.3
- Water, ultrapure
- Tris-(hydroxymethyl) aminomethane Tris, MW 121.14
- Guanidine hydrochloride, MW 95.53
- Iodoacetic acid, MW 185.9
- $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$, MW 147.0
- Sephadex G-25 pre-packed desalting columns
- 2 M NH_4OH
- 1 M HCl
- Tris-HCl*

1.4. Application

Use Trypsin recombinant, Proteomics Grade for digesting proteins prior to mass spectrometric analysis, see Figure 1.

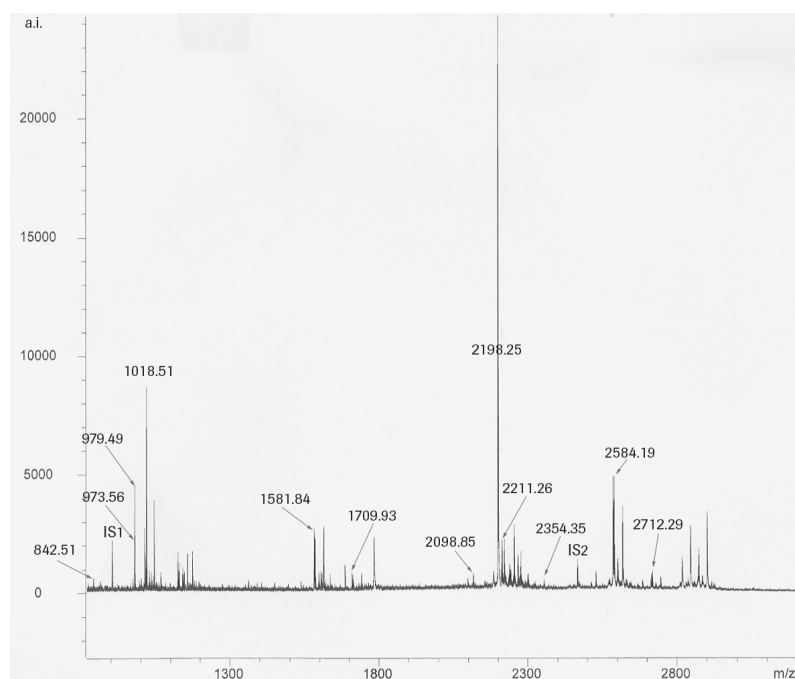


Fig. 1: MALDI-MS spectrum of bovine Carbonic Anhydrase II (EC 4.1.1.1) digested by Trypsin recombinant, Proteomics Grade. 500 ng Carbonic Anhydrase II was separated on a SDS polyacrylamide gel; the Coomassie-stained gel band containing Carbonic Anhydrase II was excised, treated, see section, **Protocols, Gel processing**, and finally digested with 50 ng Trypsin recombinant, Proteomics Grade overnight at +37°C. The extracted peptides were analyzed in reflector mode using a Bruker Daltonics Reflex III/TOF detector. Mass values indicate identified peptides using Mascot Search, IS1 resp. IS2 refer to internal standards, peaks at 842.51 and 2211.26 are due to autolysis of trypsin.

2. How to Use this Product

2.1. Before you Begin

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

Working Solution

Digestion of proteins in gels

Number	Solution	Preparation/Composition	Storage and Stability	For use in...
1	Ammonium hydrogen carbonate	<ul style="list-style-type: none"> ▪ 100 mM, pH 8.5 ▪ Dissolve 790.6 mg NH_4HCO_3 in 90 ml water, adjust the pH to 8.5 with 2 M NH_4OH, then use water to bring the final volume to 100 ml. 	–	Destaining solution, Reducing solution, Alkylating solution, and Digestion buffer.
2	Destaining solution	<ul style="list-style-type: none"> ▪ 30% acetonitrile in 100 mM NH_4HCO_3 ▪ Bring 30 ml acetonitrile to a final volume of 100 ml with 100 mM NH_4HCO_3 (Solution 1). 	–	Destaining of Coomassie-stained gel
3	Reducing solution	<ul style="list-style-type: none"> ▪ 10 mM DTT in 100 mM NH_4HCO_3 ▪ Prepare a 100 mM DTT solution by dissolving 15.4 mg DTT/ml in 100 mM NH_4HCO_3 (Solution 1), then dilute 10-fold with 100 mM NH_4HCO_3 (Solution 1). 	–	Reduction
4	Alkylating solution	<ul style="list-style-type: none"> ▪ 54 mM iodoacetamide in 100 mM NH_4HCO_3 ▪ Dissolve 10 mg/ml iodoacetamide in 100 mM NH_4HCO_3 (Solution 1). 	–	Carboxymethylation
5	Extraction solution	<ul style="list-style-type: none"> ▪ Mix 350 μl acetonitrile with 650 μl water and 4 μl TFA. 	–	Peptide extraction
6	Reconstitution solution	<ul style="list-style-type: none"> ▪ 10 mM HCl ▪ Add 100 μl 1 M HCl to 9.90 ml water, then mix. 	–	Reconstituted Trypsin Solution 8
7	Digestion buffer	<ul style="list-style-type: none"> ▪ 50 mM NH_4HCO_3 pH 8.5/5% acetonitrile ▪ Add 500 μl acetonitrile to 5 ml 100 mM NH_4HCO_3 (Solution 1), add ultrapure water to a final volume of 10 ml. 	–	Digestion mixture

2. How to Use this Product

8	Reconstituted Trypsin solution	<ul style="list-style-type: none"> Reconstitute the lyophilizate (one bottle) to a trypsin concentration of 0.1 mg/ml by adding 250 µl Reconstitution solution (Solution 6) to 25 µg Trypsin recombinant, Proteomics Grade, or Add 1 ml Reconstitution solution (Solution 6) to 100 µg Trypsin recombinant, Proteomics Grade. 	Store in aliquots 1 month at –15 to –25°C. ⚠ Avoid repeated freezing and thawing.	Trypsin digest solution (Solution 9)
9	Trypsin digest solution	Dilute Reconstituted Trypsin solution (Solution 8) to a final trypsin concentration of 0.01 mg/ml by mixing it with a 9-fold volume of Digestion buffer (Solution 7) in a separate reaction vial.	Prepare fresh.	Digestion mixture

Digestion of proteins in solution

Number	Solution	Preparation/Composition	Storage and Stability	For use in...
1	Denaturation buffer	<ul style="list-style-type: none"> 100 mM Tris, pH 8.5, 6 M guanidine HCl, 2 mM DTT Dissolve 57.3 g guanidine HCl, 1.21 g Tris, and 30.9 mg DTT in 60 ml water, adjust the pH to 8.5 with 2 M HCl and add ultrapure water to a final volume of 100 ml. <p>⚠ Guanidine HCl is harmful, handle with care.</p>	–	Denaturation of target proteins
2	Alkylating solution	<ul style="list-style-type: none"> 200 mM iodoacetic acid Dissolve 37.2 mg in 1 ml water. The solution should be colorless. <p>⚠ Keep protected from light.</p>	–	Carboxymethylation
3	Reconstitution solution	<ul style="list-style-type: none"> 10 mM HCl Add 100 µl 1 M HCl to 9.90 ml water, then mix. 	–	Reconstituted Trypsin solution (Solution 5)
4	Dilution buffer	<ul style="list-style-type: none"> 100 mM Tris, pH 8.5, 1 mM CaCl₂ Dissolve 1.21 g Tris and 14.7 mg CaCl₂ × 2 H₂O in 70 ml water, adjust the pH to 8.5 with 2 M HCl and add ultrapure water to a final volume of 100 ml. 	–	Dilution of target protein
5	Reconstituted Trypsin solution	<ul style="list-style-type: none"> Reconstitute the lyophilizate (one bottle) to a trypsin concentration of 0.1 mg/ml by adding 250 µl Reconstitution solution (Solution 3) to 25 µg Trypsin recombinant, Proteomics Grade, or Add 1,000 µl Reconstitution solution (Solution 3) to 100 µg Trypsin recombinant, Proteomics Grade. 	Store in aliquots 1 month at –15 to –25°C. ⚠ Avoid repeated freezing and thawing.	Trypsin digest solution (Solution 6)
6	Trypsin digest solution	Dilute reconstituted Trypsin solution (Solution 5) to a final trypsin concentration of 0.02 mg/ml by mixing it with a 4-fold volume of Dilution buffer (Solution 4) in a separate reaction vial.	Prepare fresh.	Digestion mixture

2.2. Protocols

Digestion of proteins in gels

For the identification of proteins in complex mixtures:

- 1 Separate proteins first by one- or two-dimensional electrophoresis.

- 2 Fix and stain according to standard or colloidal Coomassie G staining protocols.

- 3 Excise spots or bands of proteins with a scalpel, cut into pieces, and transfer to microplate wells.

- 4 Wash gel pieces; the disulfide bonds of the proteins are reduced and alkylated.

- 5 Dry gel pieces under a vacuum after the final washing steps, and rehydrate in Trypsin digest solution.
 - i* The tryptic digest is performed overnight at +37°C or +15 to +25°C.

- 6 After desalting, for example, using Zip-Tip technology, samples are ready for spotting on a MALDI target.

Gel processing

i See section, **Working Solution** for additional information on preparing solutions.

- 1 Cut the Coomassie-stained protein band out of the gel.

- 2 Cut the piece of gel into smaller pieces of approximately 1 mm² in size.

- 3 Transfer the pieces into a microplate well.

- 4 Add 100 µl ultrapure water to the well.
 - Shake 10 minutes at +15 to +25°C on a thermomixer.

- 5 Aspirate the ultrapure water with a suitable pipette.

- 6 Repeat the wash steps (Steps 4 and 5).

- 7 Add 100 µl Destaining solution (Solution 2) to the well.
 - Shake 15 minutes at +15 to +25°C on a thermomixer.

- 8 Aspirate the supernatant with a suitable pipette.

- 9 Repeat the destaining step until the Destaining solution remains colorless.
 - i* At least four times.

- 10 Add 100 µl ultrapure water to the well.
 - Shake 15 minutes at +15 to +25°C on a thermomixer.

- 11 Aspirate the water with a pipette.

- 12 Repeat the wash steps (Steps 10 and 11).

- 13 Add 100 µl acetonitrile
 - Shake 15 minutes at +15 to +25°C on a thermomixer.

2. How to Use this Product

14 Aspirate the acetonitrile.

15 Dry the sample in a microplate at 10 mbar and 15 minutes at +37°C in a vacuum concentrator.

Reduction and carboxymethylation

i See section, **Working Solution** for additional information on preparing solutions.

1 Add 20 µl Reducing solution (Solution 3) per well.
– Incubate and shake at +15 to +25°C for 5 minutes.

2 Incubate another 30 minutes at +50°C.

3 Aspirate the supernatant.

4 Add 100 µl acetonitrile per well.
– Incubate at +15 to +25°C for 15 minutes.

5 Aspirate the supernatant.

6 Add 20 µl Alkylating solution (Solution 4).
– Incubate 15 minutes.

⚠ Keep protected from light.

7 Aspirate the supernatant.

8 Add 100 µl Destaining solution (Solution 2).
– Incubate and shake at +15 to +25°C for 10 minutes.

9 Aspirate the supernatant.

10 Add 50 µl Destaining solution (Solution 2).
– Incubate and shake at +15 to +25°C for 15 minutes.

11 Aspirate the supernatant.

12 Dry the sample in a microplate at 10 mbar and 15 minutes at +37°C in a vacuum concentrator.

Digestion and peptide extraction

i See section, **Working Solution** for additional information on preparing solutions.

1 Add 20 µl of freshly prepared Trypsin digest solution (Solution 9).
– Incubate at +15 to +25°C overnight.

2 Add 20 µl Extraction solution (Solution 5).
– Incubate and shake at +15 to +25°C for 40 minutes.

3 Desalt the peptide extract with desalting/concentrating pipette tips, such as C₁₈ resin ZipTip_{scx} pipette tips Millipore, according to the manufacturer's instructions.
– Elute the peptides into a fresh microplate.

4 The sample is now ready for MALDI target preparation.

Digestion of proteins in solution

- 1 For full digestion, denature proteins under reducing conditions by adding chaotropes, such as guanidine hydrochloride or urea, or strong denaturing detergents, such as SDS followed by a disulfide reduction and alkylation of the SH groups.
 - Remove guanidine hydrochloride or urea and the excess alkylating reagent before adding trypsin via desalting or dialysis.
 - Alternatively, treat reaction mixture with an excess of dithiothreitol to neutralize the alkylating reagent, then dilute to guanidine hydrochloride or urea concentrations that are below 0.1 M or 2 M, respectively.
-
- 2 For partial digestion, incubate protein in its native state with trypsin.
 - Inactivate Trypsin by lowering the pH of the reaction below pH 4 with addition of, for example, trifluoroacetic acid, (TFA) or trichloroacetic acid (TCA) precipitation.
 - Monitor the degree of digestion by RP-HPLC.
-

Tryptic digestion

i See section, **Working Solution** for additional information on preparing solutions.

- 1 Dissolve the target protein in Denaturation buffer (Solution 1).
 - ⚠ The Denaturation buffer contains guanidium HCl, handle with care.**
-
- 2 Heat at +50°C for 60 minutes.
-
- 3 Add half the sample volume of Alkylating solution (Solution 2).
 - Incubate at +15 to +25°C for 20 minutes.
 - ⚠ Keep protected from light.**
-
- 4 Exchange buffer against Dilution buffer (Solution 4) by gel filtration using, for example, Sephadex G-25 pre-packed desalting columns (refer to the manufacturer's instructions).
 - i The protein concentration of the eluate should be higher than 0.1 mg/ml.**
-
- 5 Add the Trypsin digest solution (Solution 6).
 - i Enzyme amount should be 1/100 -1/20 of protein by weight.**
-
- 6 Incubate 2 to 18 hours at temperatures not exceeding +37°C.
-
- 7 Halting digestion (optional for partial digestion): Stop the reaction by freezing the total digestion mixture or an aliquot at –15 to –25°C.
 - Alternatively, lower the pH below pH 4 by adding TFA or TCA to a final concentration of 10%.
-
- 8 The sample is now ready for RP-HPLC/ESI MS analysis.
-

2.3. Parameters

EC-Number

EC 3.4.21.4

Molecular Weight

23,475 Da

pH Optimum

Trypsin displays maximum activity at pH 8.0 to 8.5.

Specific Activity

≥150 U/mg (Chromozym TRY)

Specificity

Trypsin recombinant, Proteomics Grade is a serine endopeptidases from the S1 family.

- It shows primary cleavage specificity for peptide bonds located at the C-terminal side of lysine and arginine.
- The catalytic efficiency (K_{cat}/K_m) for these basic side chains is at least 10^5 times greater than for other naturally occurring amino acids.
- The preference for Arg over Lys is 2- to 10-fold higher.

Stabilizers

Trypsin is stabilized by calcium ions in the mM concentration range.

3. Troubleshooting

Observation	Possible cause	Recommendation
No or incomplete in-gel digestion.	Resistance of target protein against full tryptic cleavage.	Vary trypsin:protein ratio, digestion times, and/or temperature.
	Desalting step failed, peptide not eluting from desalting/concentrating pipette tips.	Optimize desalting/concentration procedure according to the manufacturer's instructions.
	Inactivation of trypsin in reconstituted form due to multiple freeze-thaw cycles, or use of the wrong reconstitution buffer.	After first reconstitution, store trypsin in appropriate aliquots to avoid repeated freeze-thaw cycles.
MS spectra containing additional trace signals at 841.5, 2210.1, and 5500.8.	Autolytic tryptic peptides may be generated.	Can be used to calibrate MS instrument. If these signals appear to be too strong, reduce digest time and/or temperature.
Other contaminating peptides detected.	Sample contamination during handling by keratin from skin or hair.	Improve environmental conditions: <ul style="list-style-type: none"> ▪ Clean the equipment. ▪ Wear gloves. ▪ Work in a hood.

4. Additional Information on this Product

4.1. Test Principle

Background information

Trypsin is biosynthesized as a proenzyme (trypsinogen) and proteolytically activated by enteropeptidase.

- In addition, it has an “autolysis” loop that comprises residues 143–151; it is very flexible in both trypsin and trypsinogen. Cleavage of this loop at Lys 145 yields α -trypsin, which retains some catalytic activity. This and other clipped forms are present in most trypsin preparations.
- Highly purified enzyme preparation that is free of activity from other proteases, particularly chymotrypsin, which is absent from the formulation (Figure 2).

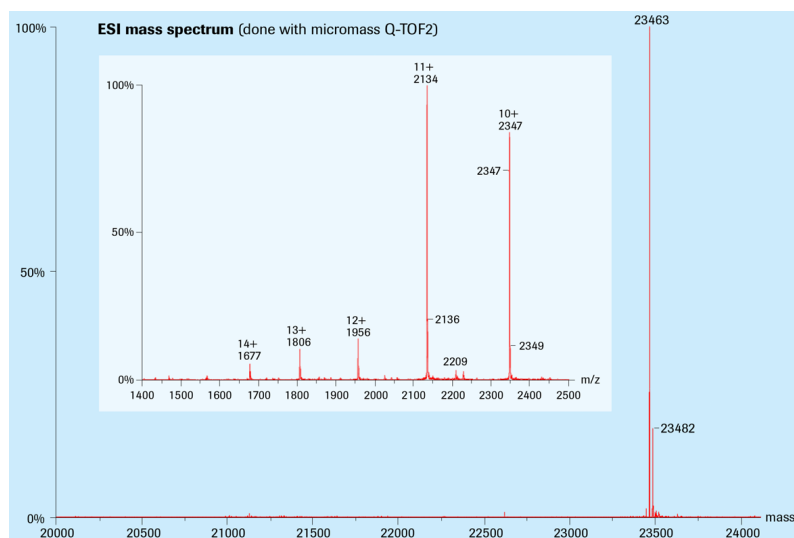


Fig. 2: Purity profile of Trypsin recombinant, Proteomics Grade (Deconvoluted spectrum, insert: raw data).

4.2. Quality Control

For lot-specific certificates of analysis, see section, **Contact and Support**.

5. Supplementary Information

5.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols

i *Information Note: Additional information about the current topic or procedure.*

⚠ Important Note: Information critical to the success of the current procedure or use of the product.

① ② ③ etc. Stages in a process that usually occur in the order listed.

① ② ③ etc. Steps in a procedure that must be performed in the order listed.

* (Asterisk) The Asterisk denotes a product available from Roche Diagnostics.

5.2. Changes to previous version

Layout changes.

Editorial changes.

Update to include new safety Information to ensure handling according controlled conditions.

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
1,4-Dithiothreitol	2 g	10 197 777 001
	10 g	10 708 984 001
	25 g	11 583 786 001
Tris hydrochloride	500 g	10 812 846 001

5.4. Trademarks

All product names and trademarks are the property of their respective owners.

5.5. License Disclaimer

For patent license limitations for individual products please refer to:

List of biochemical reagent products.

5.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

5.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

5.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site.**

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

