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

# Trypsin Sequencing Grade, modified

## from bovine pancreas

**Version: 18**
Content Version: November 2020

Lyophilized

**Cat. No. 11 418 025 001** 4 x 25 µg

**Store the product at +2 to +8°C.**

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# 1. General Information

## 1.1. Contents

| Vial / bottle | Label | Function / description | Catalog number | Content |
|---|---|---|---|---|
| 1 | Trypsin Sequencing Grade, modified | Salt-free lyophilizate. | 11 418 025 001 | 4 vials, 25 µg each |
| | | | 11 418 033 001 | 4 vials, 100 µg each |

## 1.2. Storage and Stability

### Storage Conditions (Product)

When stored at +2 to +8°C, the product is stable through the expiry date printed on the label.

| Vial / bottle | Label | Storage |
|---|---|---|
| 1 | Trypsin Sequencing Grade, modified | Store dry at +2 to +8°C. |

### Reconstitution

1. Reconstitute the lyophilizate in 1% acetic acid or 1 mM HCl.
2. Store ≤1 week at +2 to +8°C.

## 1.3. Additional Equipment and Reagent required

#### For reconstitution of lyophilizate

- 1% acetic acid or 1 mM HCl

#### For digestion of proteins in solution

*See section,* **Working Solution** *for information on preparing solutions*

- Digestion buffer
- Denaturing agents (optional)

# 2. How to Use this Product

## 2.1. Before you Begin

## General Considerations

### Precautions

The content of one vial may be used for several simultaneous digests.
- Use a new vial when repeating a digest to minimize the risk of contamination or autolysis.
- Resistance of the enzyme against autolysis in solution is remarkably increased due to modification, such as crosslinking to a hydrophilic polymer.

### Autolysis

Trypsin Sequencing Grade, modified, is more resistant to autolysis, even at pH values in the neutral and weakly basic range, therefore the enzyme can be used in high concentrations in the digestion assay. The following table shows the stability of the modified Trypsin Sequencing Grade and native trypsin in 1 mM Tris-HCl, pH 8.5 at +37°C.

| Incubation time [h] | Activity | |
|---|---|---|
| | Trypsin Sequencing Grade, modified | Trypsin Sequencing Grade, native |
| 0 | 100 | 100 |
| 2 | 100 | 48 |
| 5 | 84 | 17 |
| 20 | 34 | 2 |

### Specificity and nonspecificity of Trypsin Sequencing Grade, modified

Trypsin is a serine endopeptidase.
- At pH 7.5 to 9, it specifically hydrolyzes proteins and peptide bonds C-terminally of lysine and arginine. Amide and ester bonds of Arg and Lys are also cleaved.
- The specificity and nonspecificity of Trypsin Sequencing Grade, modified, is verified with the oxidized B-chain of insulin (insulin $B_{ox}$) as a substrate (Figures 1 and 2).

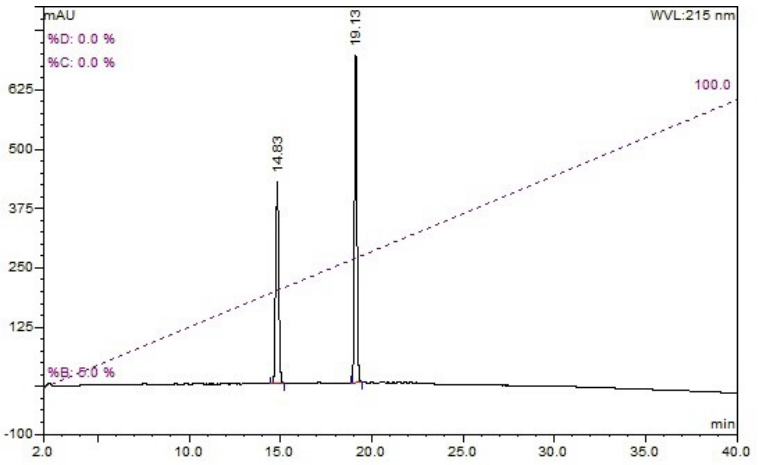


**Fig. 1:** Specificity of Trypsin Sequencing Grade, modified in reversed-phase HPLC.
High concentrations of Trypsin Sequencing Grade, modified (1 part by weight enzyme with 9 parts by weight insulin $B_{ox}$) are incubated for 1 hour to detect the fragments of the specific digested substrate.

| HPLC | Requirements |
|---|---|
| Digest | ▪ 180 µg insulin $B_{ox}$ + 10 µg Trypsin Sequencing Grade in 190 µl 100 mM Tris-HCl, pH 8.5.<br>▪ 1 hour at +37°C; reversed-phase HPLC 10 µl digest diluted with Tris buffer to 40 µl. |
| Column | Nucleosil 100-5-C18 4 × 100 mm, 5 µm |
| Solvent A | 0.1% TFA (v/v) in water |
| Solvent B | 0.1% TFA (v/v) in water; 70% acetonitrile (v/v) |
| Gradient | 40 min linearly 0 to 100% B |
| Flow rate | 1 ml/min |
| Wavelength | 215 nm |
| Fragments | 14.83 min Gly ( 23) – Lys (29)<br>19.13 min Phe (1) – Arg (22) |

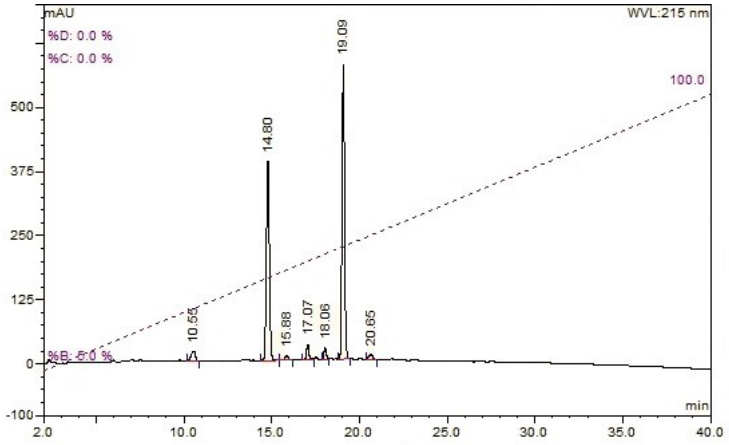


**Fig. 2:** Nonspecificity of Trypsin Sequencing Grade, modified in reversed-phase HPLC.
High concentrations of Trypsin Sequencing Grade, modified (1 part by weight enzyme with 18 parts by weight insulin $B_{ox}$) are incubated for 18 hours to detect traces of impurities.

| HPLC | Requirements |
|---|---|
| Digest | ▪ 180 µg insulin $B_{ox}$ + 10 µg Trypsin Sequencing Grade in 190 µl 100 mM Tris-HCl, pH 8.5.<br>▪ 18 hours at +37°C; reversed-phase HPLC 10 µl digest diluted with Tris buffer to 40 µl. |
| Column | Nucleosil 100-5-C18 4 × 100 mm, 5 µm |
| Solvent A | 0.1% TFA (v/v) in water |
| Solvent B | 0.1% TFA (v/v) in water; 70% acetonitrile (v/v) |
| Gradient | 40 min linearly 0 to 100% B |
| Flow rate | 1 ml/min |
| Wavelength | 215 nm |
| Fragments | 14.80 min Gly ( 23) – Lys (29)<br>19.09 min Phe (1) – Arg (22) |

# 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 buffer: 100 mM Tris-HCl*, pH 8.5

# 2.2. Protocols

## Digestion of proteins in solution

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

1. Dissolve the proteins to be sequenced in Digestion buffer.

2. For proteins that are hard to solubilize, add urea, SDS, or guanidine HCl to the Digestion buffer prior to solubilizing the protein.
   *When adding urea, also add 20 mM methylamine.*

3. To achieve a suitable concentration of the denaturing agent in the digest, the protein solution must be correspondingly diluted with buffer as shown in the table in Step 3.
   – This table shows the activity determination of Trypsin Sequencing Grade, modified with Chromozym TRY in the presence of stated concentrations of denaturing agents. Incubation of Trypsin Sequencing Grade, modified, 200 µg/ml with denaturing agent for 6 hours at +25°C in 100 mM Tris-HCl, pH 8.5.

| Denaturing agent | Concentration | Enzyme activity [%] |
|---|---|---|
| Without addition (control) | – | 100 |
| SDS (sodium dodecyl sulfate) | 0.001% (w/v) | 139 |
| | 0.01% (w/v) | 153 |
| | 0.1% (w/v) | 180 |
| Urea | 0.1 M | 170 |
| | 0.5 M | 150 |
| | 1.0 M | 100 |
| Guanidine hydrochloride | 0.1 M | 114 |
| | 0.5 M | 31 |
| | 1.0 M | 0 |
| Acetonitrile | 1% (v/v) | 260 |
| | 5% (v/v) | 270 |
| | 10% (v/v) | 310 |

4. Use an amount of enzyme that is 1/100 to 1/5 of the protein by weight.

5. Incubate 2 to 18 hours at +37°C, depending on the amount of enzyme.

# 3. Additional Information on this Product

## 3.1. Test Principle

### Preparation

Trypsin Sequencing Grade, modified is isolated from bovine pancreas as a highly purified and specific protease and subsequently modified.

## 3.2. Quality Control

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

# 4. Supplementary Information

## 4.1. 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 | |
|---|---|
| *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. |
| ❶ ❷ ❸ etc. | Steps in a procedure that must be performed in the order listed. |
| * (Asterisk) | The Asterisk denotes a product available from Roche Diagnostics. |

## 4.2. Changes to previous version

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

## 4.3. Ordering Information

| Product | Pack Size | Cat. No. |
|---|---|---|
| Reagents, kits | | |
| Tris hydrochloride | 500 g | 10 812 846 001 |

## 4.4. Trademarks

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

## 4.5. License Disclaimer

For patent license limitations for individual products please refer to:
**List of biochemical reagent products**.

## 4.6. Regulatory Disclaimer

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

## 4.7. Safety Data Sheet

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

## 4.8. 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.

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