Trypsin Sequencing Grade

From bovine pancreas

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>4 x 25 µg</th>
<th>4 x 100 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>11 418 475 001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 047 841 001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. **What this Product Does**

**Content**
Lyophilizate, salt-free.

**Storage and Stability**
Stable at +2 to +8°C until the expiration date printed on the label.

Store dry!

The working solution of Trypsin Sequencing Grade in 0.01% trifluoroacetic acid (TFA), (v/v) or 1 mM HCl may be used for maximum of one week, when stored at +2 to +8°C. Partial autolysis may occur when incubating proteins in solution at neutral to slightly basic pH-values. For this application, we recommend Trypsin Sequencing Grade.

**Application**
Trypsin Sequencing Grade is suitable for digesting proteins in solution, gels, or on blotting membranes.

2. **How to Use this Product**

2.1 **Before You Begin**

**General Handling Recommendations**
The content of one vial may be used for several simultaneous digests. A new vial should be taken when repeating a digest in order to minimize the risk of contamination or autolysis.

2.2 **Digestion of Proteins in Solution**

**Working Solution**
Reconstitute lyophilized Trypsin Sequencing Grade in 0.01% trifluoroacetic acid (TFA) (v/v), or 1 mM HCl.

**Procedure**

1. Dissolve the proteins to be sequenced in digestion buffer (100 mM Tris-HCl, pH 8.5).
2. In the case of proteins that are hard to solubilize, add urea, SDS or guanidine HCl to the digestion buffer prior to solubilizing the protein. When applying urea, Roche recommends also adding 20 mM methylamine.
3. To achieve a suitable concentration of the denaturing agent in the digest, the protein solution has to be correspondingly diluted with buffer (Table 1).
4. The recommended amount of enzyme is 1/100 to 1/20 of the protein by weight.

2.3 **Digestion of Proteins in Gels or on Blotting Membranes**

**Working Solution**
Trypsin Sequencing Grade is first dissolved with 1 mM HCl to a concentration of 0.1 mg/ml and further diluted with digestion buffer (50 mM ammonium hydrogencarbonate or 100 mM Tris-HCl, pH 8.5) to 1 - 5 µg Trypsin in 100 µl immediately before use.

In order to stabilize the trypsin calcium chloride (1 mM) can be added to the digestion buffer.

**Procedure**
Several protocols describing the cleavage of proteins in gels or on membranes have been published (1-5).

As much volume is added to the gel as every shrunk piece becomes completely reswollen and covered. For the incubation of proteins on blotting membranes, detergents such as Triton X-100 or PVP-40 are added to the digestion buffer to just completely cover the membrane piece.

A parallel control incubation using a gel or membrane piece of about the same size but without protein is recommended for each experiment. This facilitates the detection of artefacts due to the gel, membrane, or staining, as well as to a possible autolysis of the trypsin.

**Incubation Time**
The incubation time should be chosen between 2 and 18 h at +37°C depending on the amount of protein to be digested.

**Tab. 1:** Activity determination of Trypsin Sequencing Grade with Chromozym TRY in the presence of stated concentrations of denaturing agents.

<table>
<thead>
<tr>
<th>Denaturing agent</th>
<th>Concentration</th>
<th>Enzyme activity in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>without addition (control)</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>sodium dodecyl sulfate (SDS)</td>
<td>0.001% (w/v)</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>0.01% (w/v)</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>0.1% (w/v)</td>
<td>105</td>
</tr>
<tr>
<td>urea</td>
<td>0.1 M</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>0.5 M</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>1.0 M</td>
<td>90</td>
</tr>
<tr>
<td>guanidine hydrochloride</td>
<td>0.05 M</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>0.1 M</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>0.3 M</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>0.5 M</td>
<td>4</td>
</tr>
<tr>
<td>acetonitrile</td>
<td>1% (v/v)</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>5% (v/v)</td>
<td>114</td>
</tr>
<tr>
<td></td>
<td>10% (v/v)</td>
<td>134</td>
</tr>
</tbody>
</table>
3. Additional Information on this Product

3.1 Product Characteristics

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

Molecular Weight
23,500 Da

Sequence of β-trypsin

\[\text{IVDD YTCGNTVYY QVLSLGVYFH COQSLNEMQ YVSAAVCYKS} \]
\[\text{GIVQELRDRD ZNVREDGQF ISEKGSVYHP SYNNTLNNN IMLLKLEASA} \]
\[\text{SLRDEAVISIS LPTCASADT CQLSGGMN KSSQTYPFDV LECLKAPILS} \]
\[\text{DSCCSAYPD QITMNFCAQ YLEGIXDSCQ GDGGPVYCS GXLQGIVSNK} \]
\[\text{SGCQRKRFQ YYKTYCHVS MIQQTASN} \]

3.2 Quality Control

Performance and purity are checked with HPLC.

Specificity and Nonspecificity Verification
Trypsin Sequencing Grade is a serine protease that specifically cleaves peptide bonds C-terminally at lysine and arginine. The specificity and nonspecificity of Trypsin Sequencing Grade is verified using the oxidized B-chain of insulin (insulin B\textsubscript{\text{ox}}) as the substrate.

Fig. 1: Specificity of Trypsin Sequencing Grade in reversed phase HPLC. High concentrations of Trypsin Sequencing Grade (1 part by weight enzyme with 18 parts by weight insulin B\textsubscript{\text{ox}}) are incubated for 1 h to detect the fragments of the specific digested substrate.

Digest 180 \(\mu\)g insulin B\textsubscript{ox} + 10 \(\mu\)g Trypsin Sequencing Grade in 190 \(\mu\)l 100 mM Tris-HCl, pH 8.5, 1 h at +37°C; reversed phase HPLC 10 \(\mu\)l digest diluted with Tris buffer to 40 \(\mu\)l.

Column Nucleosil 100-5-C18 4 x 100 mm, 5 \(\mu\)m
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 0–100% B;
Flow rate 1 ml/min
Wavelength: 215 nm
Fragments 14.86 min Gly (23) – Lys (29)
19.11 min Phe (1) – Arg (22)

Fig. 2: Nonspecificity of Trypsin Sequencing Grade in reversed phase HPLC. High concentrations of Trypsin Sequencing Grade (1 part by weight enzyme with 18 parts by weight insulin B\textsubscript{ox}) are incubated for 1 h to detect traces of impurities of Trypsin.

Digest 180 \(\mu\)g insulin B\textsubscript{ox} + 10 \(\mu\)g Trypsin Sequencing Grade in 190 \(\mu\)l 100 mM Tris-HCl, pH 8.5, 18 h at +37°C; reversed phase HPLC 10 \(\mu\)l digest diluted with Tris buffer to 40 \(\mu\)l.

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Gradient 40 min linearly 0–100% B;
Flow rate 1 ml/min
Wavelength: 215 nm
Fragments 14.86 min Gly (23) – Lys (29)
19.12 min Phe (1) – Arg (22)

References
4. Supplementary Information

Changes to Previous Version

• Editorial changes

Text Conventions
To make information consistent and understandable, the following text conventions are used in this document:

<table>
<thead>
<tr>
<th>Text Convention</th>
<th>Use</th>
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</thead>
<tbody>
<tr>
<td>Numbered instructions</td>
<td>Stages in a process that usually occur in the order listed.</td>
</tr>
<tr>
<td>labeled 1, 2, etc.</td>
<td></td>
</tr>
</tbody>
</table>

Symbols
Symbols are used in this document to highlight important information:

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Information Note: Additional information about the current topic or procedure.</td>
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</tbody>
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Ordering Information

<table>
<thead>
<tr>
<th>Product</th>
<th>Pack Size</th>
<th>Cat. No.</th>
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<tbody>
<tr>
<td>Denaturation Reagents</td>
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</tr>
<tr>
<td>Guanidine thiocyanate</td>
<td>500 g</td>
<td>11 685 929 001</td>
</tr>
<tr>
<td>Sodium Dodecyl Sulfate</td>
<td>1 kg</td>
<td>11 667 289 001</td>
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</table>

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