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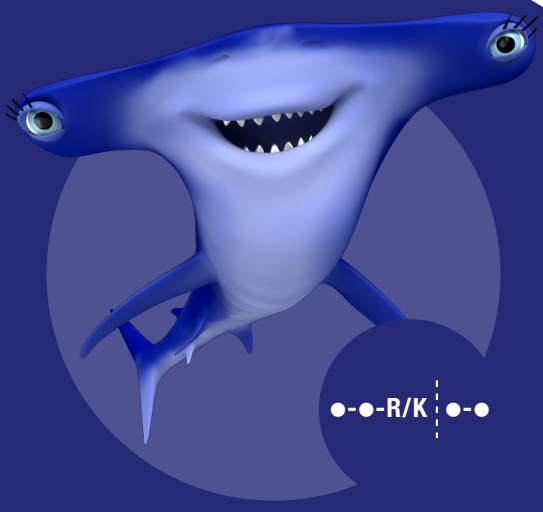

TrypsINATOR™

Lyophilized

STORE AT
-20°C

FOR RESEARCH USE ONLY

Instructions for Use

TrypsINATOR™ Lyophilized 100µg (B0-TN1-100)
Process 2–10 mg protein

DOWNLOAD INSTRUCTIONS FOR USE
www.genovis.com/ifu-B0-TN1

www.genovis.com

Lyophilized Enzyme for Arginine- and Lysine-specific Protein Digestion

TrypsINATOR (Trypsin) is a serine protease that digests proteins C-terminally of arginine and lysine residues.

TrypsINATOR is reductively methylated at lysine residues to minimize autoproteolysis, increase stability and ensure consistent performance, even during extended incubations. TrypsINATOR Lyophilized is compatible with standard digestion buffers, including Tris-HCl, ammonium bicarbonate, HEPES, PBS and TEAB. It is optimally active in a pH range of 7.0–9.0.

The TrypsINATOR enzyme has a molecular weight of 24 kDa.

CONTENT AND STORAGE

TrypsINATOR Lyophilized is supplied lyophilized in 50 mM HEPES, 100 mM NaCl, 2% trehalose, pH 8.5, with no preservatives added.

TrypsINATOR Lyophilized is shipped cold, and should be stored at -20°C upon arrival. After reconstitution, TrypsINATOR Lyophilized is stable for 4 weeks at -20°C or for 1 week at +4-8°C.

TrypsINATOR Lyophilized is for R&D use only.

QUALITY CONTROL

TrypsINATOR Lyophilized is tested to meet the specifications and lot-to-lot consistency.

0.02 µg TrypsINATOR Lyophilized digests ≥ 90% of 1 µg apomyoglobin when incubated in 50 mM Tris-HCl pH 8.0 at RT, for 30 minutes.

YOU MIGHT ALSO BE INTERESTED IN

TrypsINATOR™ LysCERATOR Mix Lyophilized
Lyophilized enzyme mix for arginine- and lysine-specific protein digestion

LysCERATOR™
Lysine-specific protein digestion

GingisREX™
Arginine-specific protein digestion

Preparations

Important Information
In general, to obtain optimal digestion, proteins require efficient solubilization, denaturation and disulfide bond reduction (with subsequent alkylation). The protocol in this instruction is provided as a guideline to facilitate digestion with TrypsINATOR. Optimization of the protocol might be necessary depending on the substrate.

Additional Materials Required

- Reaction buffer: 50 mM Tris-HCl, pH 8.0[1]
- Denaturing agent: urea
- Reducing agent (DTT)
- Alkylating agent (iodoacetamide)
- Trifluoroacetic acid or formic acid to stop enzymatic reaction

Preparation of 9 M Urea
Dissolve 270 mg urea (MW 60.06 g/mol) in 260 µl reaction buffer. Vortex vigorously and adjust the volume to 500 µl with reaction buffer. Use urea freshly prepared to avoid carbamylation. This protocol is scalable if more than 500 µl 9 M urea is required.

Preparation of 1 × 100 µg TrypsINATOR™ Lyophilized
Centrifuge the TrypsINATOR Lyophilized vial and make sure that all lyophilized material is in the bottom of the vial. Reconstitute the enzyme by adding 200 µl ddH_2O to a concentration of 0.5 mg/ml. Make sure that all lyophilized material is dissolved. Store on ice at 4°C until ready to use. For storage at -20°C, aliquot before freezing to avoid freeze-thaw cycles.

1. Optimal pH is between 7.0–9.0. TrypsINATOR is compatible with standard buffers, including Tris-HCl, ammonium bicarbonate, HEPES, PBS and TEAB.

Arginine- and Lysine-specific Protein Digestion

Sample Preparation
Dissolve the protein in the reaction buffer.[1]

1. Solubilization/Denaturation/Disulfide Reduction

1.1 In the chosen buffer, mix reagents to a final concentration of:

- ≥1 mg/ml protein.
- 4–8 M urea for denaturation.
- 5 mM DTT for reduction.

1.2 Incubate for 1 h at 37°C.

2. Alkylation

2.1 Cool down reaction to room temperature (RT) and alkylate free sulfhydryls with iodoacetamide to a final concentration of 15 mM.

2.2 Incubate for 30 min at RT in the dark.

3. Quench Iodoacetamide

3.1 To avoid overalkylation, quench excess iodoacetamide with DTT to a final concentration of 10 mM.

3.2 Incubate for 15 min at RT in the dark.

4. Sample Dilution

4.1 Dilute the sample with reaction buffer to ≤1 M of urea.

5. Add TrypsINATOR

5.1 Add TrypsINATOR in an enzyme:substrate ratio of 1:100 to 1:20.

6. Enzymatic Reaction

6.1 Incubate for 2–18 h at 37°C.[2]

6.2 The reaction can be stopped by addition of trifluoroacetic acid to a final concentration of 1%, or formic acid to a final concentration of 2%.

2. A higher enzyme:substrate ratio can facilitate shorter incubation times. This requires optimization.

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