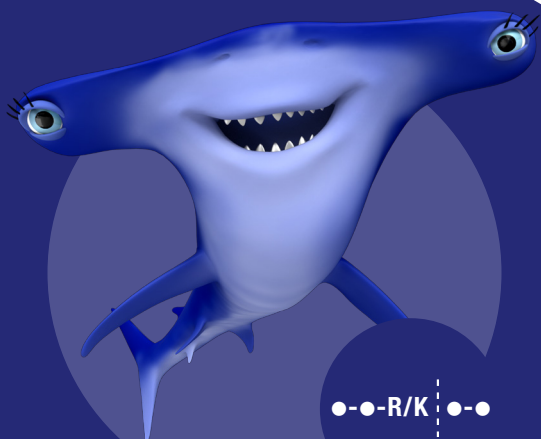




TrypsINATOR™

LysCERATOR Mix Lyophilized

STORE AT

-20°C



FOR RESEARCH USE ONLY

Instructions for Use

TrypsINATOR™ LysCERATOR Mix
Lyophilized 100 µg (B0-TL1-100)
Process 2–10 mg protein

DOWNLOAD INSTRUCTIONS FOR USE



www.genovis.com/ifu-B0-TL1

Lyophilized Enzyme Mix for Arginine- and Lysine-specific Protein Digestion

TrypsINATOR (Trypsin) is a serine protease that digests proteins C-terminally of arginine and lysine residues. By combining TrypsINATOR with LysCERATOR, the TrypsINATOR LysCERATOR Mix offers enhanced digestion efficiency at lysine residues to further reduce missed cleavages, while offering improved digestion at challenging lysine-proline (K-P) sites.

TrypsINATOR LysCERATOR Mix Lyophilized is compatible with standard digestion buffers, including Tris-HCl, ammonium bicarbonate, HEPES, PBS and TEAB. It is active in a pH range of 6.0–10.0.

The TrypsINATOR enzyme has a molecular weight of 24 kDa and the LysCERATOR enzyme 29 kDa.

CONTENT AND STORAGE

TrypsINATOR LysCERATOR Mix Lyophilized is supplied lyophilized in 50 mM HEPES, 100 mM NaCl, 3.8 mM CaCl_2 , 2% trehalose, pH 8.5, with no preservatives added.

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

TrypsINATOR LysCERATOR Mix Lyophilized is for R&D use only.

QUALITY CONTROL

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

0.01 μg TrypsINATOR LysCERATOR Mix Lyophilized digests $\geq 90\%$ 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™ Lyophilized

Lyophilized enzyme 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 LysCERATOR Mix. Optimization of the protocol might be necessary depending on the substrate.

Additional Materials Required

- Reaction buffer: 50mM Tris-HCl, pH 8.0¹
- Denaturing agent: urea
- Reducing agent (DTT)
- Alkylating agent (iodoacetamide)
- Trifluoroacetic acid or formic acid to stop enzymatic reaction

Preparation of 9M 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 LysCERATOR Mix Lyophilized

Centrifuge the TrypsINATOR LysCERATOR Mix Lyophilized vial and make sure that all lyophilized material is in the bottom of the vial. Reconstitute the enzyme by adding 200 µl ddH₂O 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 6.0–10.0. TrypsINATOR LysCERATOR Mix 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 LysCERATOR Mix

- 5.1 Add TrypsINATOR LysCERATOR Mix 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.

TrypsINATOR™ LysCERATOR Mix Lyophilized

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