GENOVIS

LysCERATOR™

Lyophilized

STORE AT
-20°C

FOR RESEARCH USE ONLY

Instructions for Use

LysCERATOR™ Lyophilized 20 µg (B0-LC1-020)
Process 0.4–2 mg protein

LysCERATOR™ Lyophilized 5 × 20 µg (B0-LC1-100)
Process 5 × 0.4–2 mg protein

DOWNLOAD INSTRUCTIONS FOR USE
www.genovis.com/ifu-B0-LC1

Lyophilized Enzyme for Lysine-specific Protein Digestion

LysCERATOR (Lys-C) is a lysine-specific endopeptidase that digests proteins C-terminally of lysine residues, including at lysine-proline linkages.

LysCERATOR Lyophilized is active in a pH range of 7.0–9.0 and retains high activity under denaturing conditions up to 8 M urea and 2 M guanidine hydrochloride.

The LysCERATOR enzyme has a molecular weight of 29 kDa.

CONTENT AND STORAGE

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

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

LysCERATOR Lyophilized is for R&D use only.

QUALITY CONTROL

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

0.01 µg LysCERATOR Lyophilized digests ≥ 90% of 1 µg human IgG1 when incubated in 100 mM Tris pH 8.0 including 4 M Urea at 37°C, for 2 hours.

YOU MIGHT ALSO BE INTERESTED IN

GingisREX®
Arginine-specific protein digestion

Preparations

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

Additional Materials Required

- Reaction buffer: 0.1 M Tris-HCl, pH 8.0[1].
- Denaturing agent (urea or guanidine hydrochloride (Gd-HCl)).
- 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, and if possible (depending on the protein to be digested), keep the temperatures in reactions with urea around 30°C to avoid carbamylation. This protocol is scalable If more than 500 µl 9 M urea is required.

Preparation of 6 M Guanidine Hydrochloride
Dissolve 287 mg Gd-HCl (MW 95.53 g/mol) in 260 µl reaction buffer. Vortex vigorously and adjust the volume to 500 µl with reaction buffer. If higher concentrations of Gd-HCl is desired, heating for longer periods may be required, or pre-prepared solutions can be bought from various vendors.

Preparation of 1 × 20 µg LysCERATOR™ Lyophilized
Centrifuge the LysCERATOR Lyophilized vial and make sure that all lyophilized material is in the bottom of the vial. Reconstitute the enzyme by adding 40 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.

1. Optimal pH is between 7.0–9.0. Compatible buffers are Tris-HCl and HEPES.

Lysine-specific Protein Digestion

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

1. Solubilization/Denaturation/Disulphide Reduction

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

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

1.2 Incubate for 1 h at 30-37°C.[2]

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 ≤2 M of denaturing agent[3].

5. Add LysCERATOR

5.1 Add LysCERATOR in an enzyme:protein ratio of 1:100 to 1:20.

6. Enzymatic Reaction

6.1 Incubate for 1–18 h at 37°C.

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. Depending on the protein substrate, a shorter or longer heating time may be required to solubilize and denature the protein prior to incubation. Heating at 30°C is suggested for denaturation in urea to reduce the formation of protein carbamylation.
3. For denaturation in urea, this is to reduce the formation of protein carbamylation. For denaturation in Gd-HCl this is to improve digestion efficacy.

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