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Product Information

Alpha-lytic protease
from bacterial source

Catalog Number **A6362**
Storage Temperature –70 °C

TECHNICAL BULLETIN

EC 3.4.21.12
Synonyms: aLP, WT aLP

Product Description
Alpha-lytic protease (aLP) is a protease with alternative specificity compared to such proteases as trypsin for proteomics applications. It cleaves after T, A, S, and V residues. This specificity makes aLP an alternative protease useful for proteomics applications and it generates peptides of similar average length as trypsin.

aLP was first isolated from the myxobacterium *Lysobacter enzymogenes*.[1] The pro-form of aLP contains 397 amino acids and the role of the pro-region in the activation, secretion, and folding of aLP has been studied.[2] The methionines at positions 190 and 213 are responsible for the particular specificity toward peptide substrates with small hydrophobic side chains at the P_1 position. Mature aLP contains 198 amino acids[2] and several studies have been published on the active site and catalytic mechanism.[6,7] Crystal structures of aLP have been reported[4,5] and the tertiary structural core resembles those of pancreatic serine proteases.[3]

Molecular mass: 19,860 Da (mature form)

Optimal pH: 5.0 (storage), 7.5 (activity)

Isoelectric point (pI): 9.69

Gene ID or Accession Number: 150130 (pre-pro form)
GeneBank: AAA25409.1 (pre-pro form)

This aLP product is supplied in a solution of 10 mM sodium acetate buffer, pH 5.0.

Specific activity: ≥0.0005 unit/mg-P

Unit definition: One unit of aLP will produce one mmole of pNA per minute per mg protein from 0.5 mM N-succinyl-Ala-Ala-Ala-PNA at 25 °C in 100 mM Tris-HCl, pH 7.5.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
The product ships on dry ice and long-term storage at –70 °C is recommended.

The aLP enzyme should be stored in 10 mM sodium acetate buffer, pH 5.0.

Procedures
aLP activity is enhanced ~1.75-fold in the presence of 0.1% sodium deoxycholate. aLP activity is inhibited under the following conditions:

- 1.0% sodium deoxycholate: ~60% activity
- 0.1% sodium dodecyl sulfate (SDS): ~50% activity
- 1.0% SDS: ~40% activity
- 1 M guanidine hydrochloride: ~20%
- 4 M guanidine hydrochloride: ~1% activity, essentially inactivated

A. Solution Digestion
For peptide or protein digestion, a ratio between 1:100 to 1:20 (w/w) of enzyme to substrate is recommended. The peptide or protein substrate can be dissolved in 100 mM ammonium bicarbonate, pH 8.5 or 100 mM Tris-HCl, pH 8.5. For MALDI analysis, ammonium bicarbonate buffer should be used, as Tris-HCl buffer is incompatible with MALDI analysis.

Upon addition of the aLP solution to the substrate protein solution, the recommended incubation time is between 2–18 hours at 37 °C, depending on the enzyme-to-substrate ratio.

B. In-gel Digestion

aLP may also be used for in-gel protein digestions with subsequent identification by mass spectrometry. References for digestion protocols from gels or on membranes have been published.[8-15]

The following procedure starts with a Coomassie Brilliant Blue, SYPRO® Orange, or SYPRO Ruby stained 1D or 2D polyacrylamide gel. For silver-stained gels, a gel destaining step different than that used for dye-stained gels is required. The ProteoSilver™ Plus Silver Staining Kit (Catalog Number PROTSIL2) is recommended for silver staining prior to enzymatic digestion and MS analysis. PROTSIL2 contains destaining solutions for silver-stained gels and a procedure to prepare gel slices for aLP digestion.

1. Carefully cut the band of interest from a 1D gel or the protein spot from a 2D gel, using a scalpel or razor blade, taking care to include only stained gel. Lift the gel piece out with clean flat-nosed tweezers.

2. Place the gel piece in a siliconized microcentrifuge tube or equivalent.
   Notes: A siliconized tube reduces binding of the peptides to the tube surface. In the case of downstream MALDI analysis, if unsure of chemicals leaching from the tube, which could interfere with or suppress the MALDI-MS signal, prewash the tube with 100 µL of a 0.1% trifluoroacetic acid (TFA) in 50% acetonitrile solution and then allow it to dry before use.

   The gel piece may be cut into equal sections of 1–1.5 mm size and the sections may be used in place of the intact piece.

3. Cover the gel piece with 200 µL of 200 mM ammonium bicarbonate with 40% acetonitrile, and incubate at 37 °C for 30 minutes. Remove and discard the solution from the tube.

4. Repeat step 3 again.

5. Dry the gel piece in a Speed Vac® for 15–30 minutes.

6. Add 0.4 µg of aLP to the gel sample.

7. Add 50 µL of 40 mM ammonium bicarbonate in 9% acetonitrile solution to the gel sample.

8. Confirm that the gel piece is at the bottom of the tube and covered with liquid.

9. Incubate for 4 hours to overnight at 37 °C.
   Note: A shorter digestion time may be sufficient, but may yield slightly lower sequence coverage.

10. After the incubation, remove the liquid from the gel piece and transfer the liquid to a new labeled tube. This solution contains the extracted aLP-digested peptides. If MALDI analysis is to be performed at this step, acidification with TFA prior to matrix addition may be needed.

11. Add 50 µL of a 0.1% TFA in 50% acetonitrile solution to the gel piece. Incubate for 30 minutes at 37 °C.
    Note: This extraction step is expected to increase the peptide yield by ~5%, by analogy with trypsin.[15] At the user's discretion, step 11 may be omitted and the sample solution from step 10 analyzed directly.

12. Remove the 0.1% TFA in 50% acetonitrile solution and combine with the liquid from step 10.

13. The combined sample solution from step 12 is ready for MALDI-MS analysis.

References

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