

Product Information

Alpha-lytic protease, M190A mutant

from bacterial source
recombinant, expressed in *E. coli*

Catalog Number **A6487**

Storage Temperature $-70\text{ }^{\circ}\text{C}$

TECHNICAL BULLETIN

Synonym: M190A aLP

Product Description

Alpha-lytic protease (aLP) is a protease with alternative specificity compared to such proteases as trypsin for proteomics applications. Wild-type (WT) aLP cleaves after T, A, S, and V residues.^{1,2} The M190A (Met¹⁹⁰ → Ala¹⁹⁰) mutant of aLP has different cleavage specificities and cleaves after M, F, and L residues in addition to T, A, S, and V residues. Both the WT and M190A forms of aLP generate peptides of similar average length as trypsin.

In WT aLP, the methionines at positions 190 and 213 are responsible for the particular specificity toward peptide substrates with small hydrophobic side chains at the P₁ position. The M190A mutation results in the ability to cleave peptide substrates which have larger hydrophobic side chains at the P₁ position with greater efficiency compared to WT aLP, in addition to accommodating peptides with small hydrophobic side chains, as seen with WT aLP.^{4,5} Other structural changes in M190A aLP compared to WT aLP have been discussed.^{5,6}

Molecular mass: 19,800 Da (mature form)

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

Isoelectric point (pI): 9.69

This M190A aLP recombinant product is expressed in *E. coli* and is supplied in a solution of 10 mM sodium acetate buffer, pH 5.0.

Specific activity: ≥ 0.05 unit/mg-P

Unit definition: One unit of M190A 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 Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The product ships on dry ice and long-term storage at $-70\text{ }^{\circ}\text{C}$ is recommended.

The M190A aLP enzyme should be stored in 10 mM sodium acetate buffer, pH 5.0.

Procedure

M190A aLP activity is enhanced ~1.4-fold in the presence of 0.1% sodium deoxycholate and nearly full activity is retained in the presence of 1.0% sodium deoxycholate. M190A aLP activity is inhibited under the following conditions:

- 0.1% sodium dodecyl sulfate (SDS): ~40% activity
- 1.0% SDS: ~30% 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 M190A 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

M190A 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.⁷⁻¹⁴

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 M190A 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 μ L 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 μ L 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 μ g of M190A aLP to the gel sample.
7. Add 50 μ L of 40 mM ammonium bicarbonate in 9% acetonitrile solution to the gel sample.
8. Confirm 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 M190A 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 μ L 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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