

Product Information

Mucinase StcE

Mass spec suitable, from EHEC, recombinant, powder

SAE0202

Synonyms: Secreted protease of C1 esterase inhibitor, Mucin-specific protease from enterohemorrhagic *Escherichia coli* O157:H7

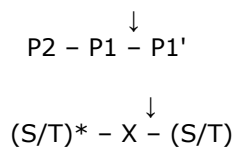
Storage Temperature -20 °C

Product Description

Mucins are a family of high molecular weight, heavily glycosylated proteins that are produced by epithelial tissues in most animals.^{1,2} Mucin domains are notable for their high frequency of Ser (S) and Thr (T) residues which are *O*-glycosylated with α -*N*-acetylgalactosamine (α -GalNAc). This leads to dynamic and very heterogenous glycoprotein populations which cannot be predicted only from genomic information.³ Mucins can contain hundreds to thousands of amino acids, and consist of >50% glycosylation by mass.⁴

Mucin-domain glycoproteins participate in many biological processes. Mucin domains are present throughout the human body and are relevant to biological processes such as embryogenesis,⁵ barrier formation,⁶ host-pathogen interactions,⁷ and immune signaling.⁸ Mucins are also used as biomarkers for conditions such as ovarian cancer and lung cancer.⁹ The stiff, elongated, and highly hydrated structures of mucin domains render them as important modulators of cell-level and protein-level biophysics.¹⁰

Investigating biological functions of mucins at the molecular scale is a challenge, as few tools are available to probe mucin domains. Secreted protease of C1 esterase inhibitor (Mucinase StcE) is a bacterial metalloprotease from *Escherichia coli* that can be used to digest densely *O*-glycosylated mucins (see Figure 1). Mucinase StcE cleaves mucin domains by recognizing the following consensus P2 – P1 – P1' sequence:



The * of the (S/T)* at the P2 site represents an occupied glycosylation site. Figure 2 depicts the consensus sequence.¹¹

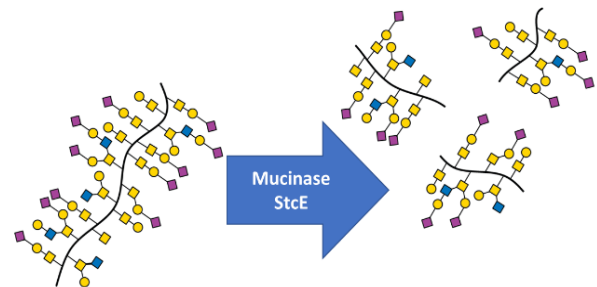


Figure 1 Mucinase StcE is a highly active protease on densely glycosylated mucins. It will fragment mucins into smaller glycopeptides similarly to schematic above.¹¹

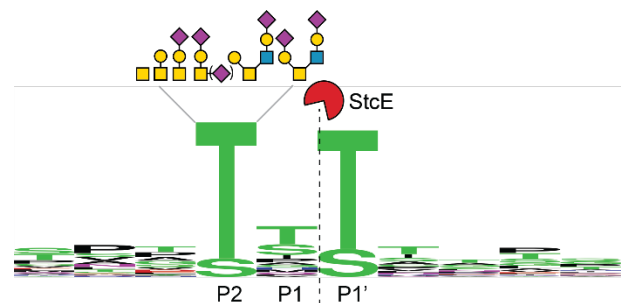


Figure 2 Depiction of consensus sequence and specificity.¹¹

Mucinase StcE cleaves on the amino side of the P1' (second S or T) site. The P1 site (X) can be any, or no, amino acid. In the case of no amino acid at the X site, the efficiency of Mucinase StcE cleavage will be lower.¹¹ The P2 position (first S or T) is always observed to be occupied by an O-linked glycan that has a O-GalNAc core. The other two amino acid sites, P1 and P1', may also contain glycan groups, which do not interfere with Mucinase StcE activity.

Mucinase StcE is active at: ¹¹

- pH range of 6.1 – 9.0
- temperature range of 4 – 55 °C
- high salt (up to 500 mM NaCl)
- with detergents (saponin, digitonin, SDS)

Reagent

This product is a purified recombinant enzyme expressed in *E. coli* with a His-Tag®, and is supplied as a lyophilized powder. The product is tested for suitability by digestion of a recombinant mucin protein.

Storage/Stability

Store this product at –20 °C (range of –25 °C to –10 °C). The product retains activity for at least 2 years when stored lyophilized at –20 °C.

Precautions and Disclaimer

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.

Preparation Instructions

Solutions of Mucinase StcE can be prepared by reconstitution of the lyophilized 100 µg vial contents in 200 µL of either water or PBS.

- The resuspended protein solution is stable for 2 weeks at 2-8 °C.
- For longer storage, aliquot the protein solution and store at -20 °C.
- The enzyme solution should be protected from direct light.

Procedure

The following is a sample procedure for MS analysis of mucin proteins.¹¹

Step 1: Mucinase StcE digestion

1. Digest sample with Mucinase StcE in a ratio of 1:10 Mucinase StcE:Sample for 3 hours at 37 °C, in 50 mM ammonium bicarbonate (such as Catalogue Number A6141).
2. For maximal activity it is recommended, to use a surfactant, such as ProteaseMAX™ or RapiGest™, at 0.1%.

Step 2: Alkylation

1. Dilute sample ~6-fold with 50 mM ammonium bicarbonate.
2. Add DTT to a concentration of 5 mM.
3. Incubate at 56 °C for 20 minutes.
4. Cool to room temperature.
5. Add iodoacetamide to a concentration of 15 mM.
6. Incubate at room temperature for 15 minutes.

Step 3: Trypsin digestion

1. The digestion is completed by adding sequencing-grade trypsin in a 1:20 Trypsin:Sample ratio, for 8 hours at 37 °C.
2. Reaction is quenched by adding formic acid to a concentration of 0.3%.

Downstream LC-MS/MS

- The sample should be processed by reverse phase (RP) clean-up, such as with a C18 column or a ZipTip®, before subjecting the sample to MS analysis.
- An OrbiTrap™ analyzer coupled to HPLC will work well for high resolution analysis.

References

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