

## 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

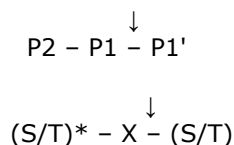
Storage Temperature -20 °C

## Product Description

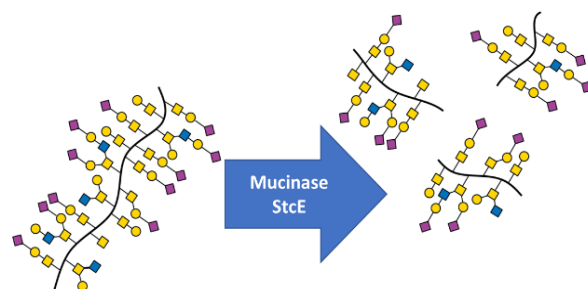
Mucins are a family of high molecular weight, heavily glycosylated proteins that are produced by epithelial tissues in most animals.<sup>1,2</sup> Mucin domains are notable for their high frequency of Ser (S) and Thr (T) residues which are O-glycosylated with α-N-acetylgalactosamine (α-GalNAc). This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

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,<sup>5</sup> barrier formation,<sup>6</sup> host-pathogen interactions,<sup>7</sup> and immune signaling.<sup>8</sup> Mucins are also used as biomarkers for conditions such as ovarian cancer and lung cancer.<sup>9</sup> The stiff, elongated, and highly hydrated structures of mucin domains render them as important modulators of cell-level and protein-level biophysics.<sup>10</sup>

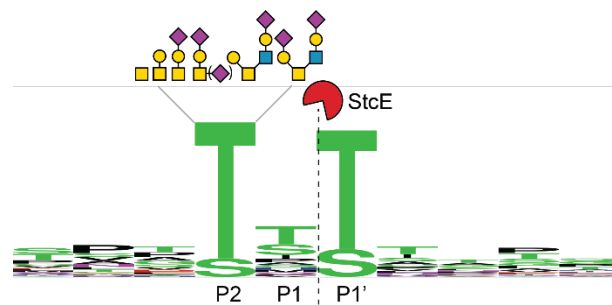
Investigating biological functions of mucins at the molecular scale is a challenge, as few tools are available to probe mucin domains. Secreted protease of C1 esterase inhibitor (Mucinase StcE) is a bacterial metalloprotease from *Escherichia coli* that can be used to digest densely O-glycosylated mucins (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.<sup>11</sup>



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



**Figure 2** Depiction of consensus sequence and specificity.<sup>11</sup>

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.<sup>11</sup> 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: <sup>11</sup>

- 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.<sup>11</sup>

### 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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