Product Information

# Mucinase SmE

Recombinant, Mass Spec Suitable

**SAE0220**

Synonyms: SmEnhancin from *Serratia marcescens*, Recombinant, Liquid

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) linked glycans. This leads to dynamic and very heterogenous glycoprotein populations which cannot be predicted from genomic information alone.[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. *Serratia marcescens* Enhancin (SmE) has a unique ability to cleave peptide bonds near glycosylated sites that would otherwise be unavailable to proteases like Trypsin or Lys-C. This enhances glycosylation site and glycoform analysis by MS. Mucinase SmE has the ability for selectively cleave along the mucin glycoprotein backbone. Unlike other mucinases, Mucinase SmE harbors the unique ability to cleave at residues bearing extremely complex glycans which enables improved mass spectrometric analysis of several mucins, including the entire TIM family.

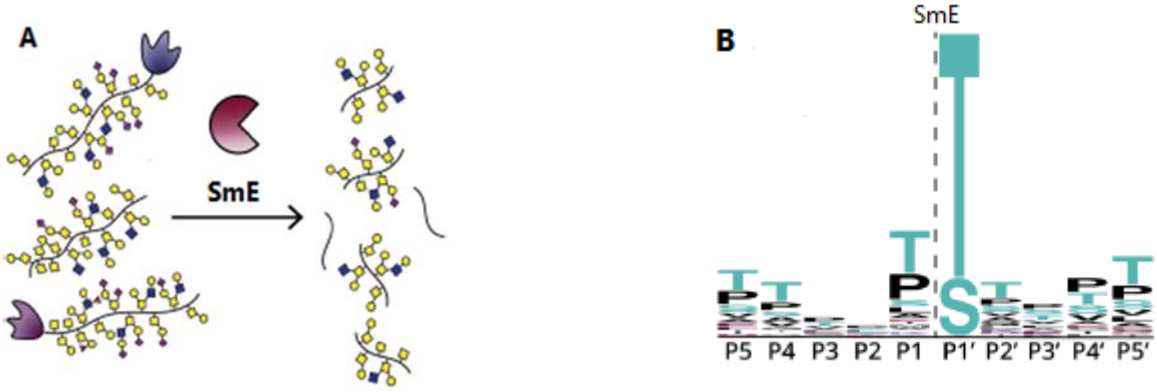


**Figure 1**: (**A**) Mucinase SmE is a highly active protease on densely glycosylated mucins. (**B**) Depiction of consensus sequence and specificity.[11]

Mucinase SmE cleaves N-terminally to a glycosylated Ser or the residue, Figure 1 (**B**) depicts the consensus sequence.[11] The recognized site accommodates a variety of *O*-linked glycans at the P1' position, including sialylated core 1 and core 2 *O*-linked glycans as well as fucosylated ABO blood group antigens. When benchmarked against other proteases known to target *O*-glycoproteins like OgpA and ImpA, SmE has been shown to perform better in terms of tolerance for site occupancy and glycoform complexity.[11]

Mucinase SmE provides a complementary cleavage profile to Mucinase StcE (SAE0202). Contrary to StcE digestion that requires an additional tryptic digest prior to MS peptide analysis, when using Mucinase Sme analysis can be done with SmE alone. It is recommended to perform both options to obtain maximal coverage.

## Reagent

This product is a purified recombinant enzyme expressed in *E. coli* with a His-Tag® and is supplied as a liquid solution. 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 frozen 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

Thaw the Mucinase SmE by brief incubation at 37 °C.

- The 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 SmE digestion

1. Digest sample with Mucinase SmE in a ratio of 1:10 SmE:Sample for 16 hours at 37 °C, in 50 mM ammonium bicarbonate (such as A6141).
2. For maximal activity it is recommended, to use a surfactant, such as ProteaseMAX™ or RapiGest®, at 0.1%.

### Step 2: Alkylation

1. Add DTT to a concentration of 2 mM.
2. Incubate at 65 °C for 20 minutes.
3. Cool to room temperature.
4. Add iodoacetamide to a concentration of 5 mM.
5. Incubate in dark at room temperature for 15 minutes.

### Step 3: Trypsin digestion (optional)

1. The digestion can be completed by adding sequencing-grade trypsin in a 1:50 Trypsin:Sample ratio, for 6 hours at 37 °C. It is advised to analyze the sample with and without the additional trypsin digest and compare results.
2. Reaction is quenched by adding formic acid to a concentration of 0.3%.

### Downstream LC-MS/MS

The sample should be diluted prior to desalting using 1% TFA until the sample is acidified. Sample extraction can be done by using Empore™ Extraction Disk solid phase extraction (SPE) Cartridge or equivalent before subjecting the sample to MS analysis.

- If digestion is done only using Mucinase SmE, it is recommended to first desalt sample using small pore reverse-phase SPE, or low MW filter to remove intact proteins/large protein fragments from the sample. It is recommended to use large pore columns such as Chromolith®CapRod® RP Monolithic Capillary column or equivalent, as the separation column pre-detector.

  *O*-glycosylation is best analyzed using mass spectrometry instruments capable of ETD/ECD/EAD/UVPD and similar fragmentation methods capable of fragmenting only the peptide backbone while leaving the glycan chains intact or partially fragmented.

- Instrument methods should be tailored for the software used for the data analysis. Some software solutions require non-trivial MS data-acquisition methods (such as O-pair).[12]

## References


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