

Product Information

Mucin Probe, StcE

Biotin and FLAG® tagged, powder

SAE0212

Product Description

Synonyms: Catalytically inactive Mucin specific protease StcE

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 serine (Ser, S) and threonine (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

The Mucin Probe is a proteolytically inactive E447D mutant of Mucinase StcE that maintains ability to tightly bind mucin proteins, without digesting the polypeptide bond.¹¹ This makes it a useful probe for targeting or labelling mucinous tissues for histological microscopy, immunoassay and other techniques where selective binding to mucin is required. For convenience in removal, tagging, and assay development, the recombinant protein carries a 3xFLAG® tag and is enzymatically biotinylated at the C-terminus of the protein.

Reagent

This product is a purified recombinant enzyme expressed in *E. coli* as a biotinylated protein with a His-Tag® and a 3xFLAG® tag. It is supplied as a lyophilized powder. The product is tested for suitability by binding to a recombinant mucin protein.

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

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.

Preparation Instructions

Solutions of Mucin Probe can be prepared by reconstitution of the lyophilized 200 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 Mucin Probe can be used for the following applications:

- Immunohistochemistry
- Immunoblotting
- Flow cytometry
- Pulldowns

The recommended Mucin Probe concentration for most application is between 10 to 40 $\mu\text{g}/\text{mL}$ and should be calibrated per application and sample type.

For all applications, it is required to use a secondary reagent to mediate detection or capture. Depending on the application, this can be achieved with either:

- Conjugated anti-FLAG[®] antibodies (Cat. Nos. A8592, P2983, A4596, M8823, A9594, F4049)
- Conjugated streptavidin/avidin based reagents (Cat. Nos. A3402, S1638, OR03L, 18-152, STREPMAG-RO)

Immunohistochemistry Application Results

Examples of results from microscopy and FACS can be seen in Figures 1, 2 and 3.

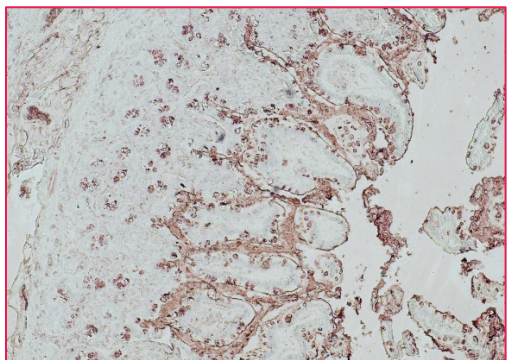


Figure 1. Human colon imaging with 10 $\mu\text{g}/\text{mL}$ mucin probe (Cat. No. SAE0212) and 20 $\mu\text{g}/\text{mL}$ anti-FLAG[®]-HRP (Cat. No. A8592).

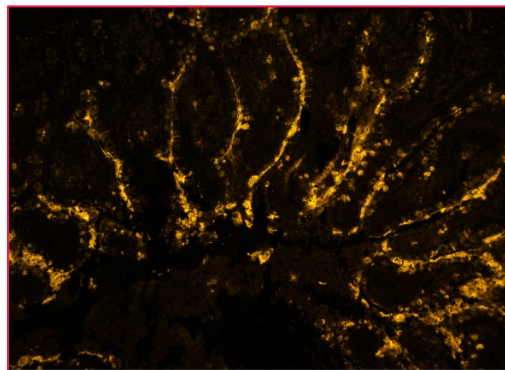


Figure 2. Human intestine immunohistochemistry with 10 $\mu\text{g}/\text{mL}$ mucin probe (Cat. No. SAE0212) and 10 $\mu\text{g}/\text{mL}$ Streptavidin-R-PE (Cat. No. A3402).

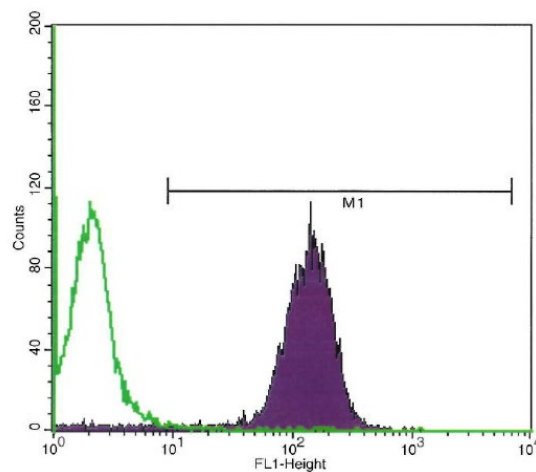


Figure 3. FACS analysis of HeLa cells (1 million cells per test). Test (purple) uses 1 $\mu\text{g}/100 \mu\text{L}$ reaction of mucin probe (Cat. No. SAE0212) and 1:100 monoclonal ANTI-FLAG[®] M2-FITC (Cat. No. F4049). Negative control (green) uses only 1:100 monoclonal ANTI-FLAG[®] M2-FITC.

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References

1. Marin, F. *et al.*, *Curr. Top. Dev. Biol.*, **80**, 209-276 (2008).
2. Hang, H.C., and Bertozzi, C.R., *Bioorg. Med. Chem.*, **13(17)**, 5021-5034 (2005).
3. Woo, C.M. *et al.*, *Nat. Methods*, **12(6)**, 561-567 (2015).
4. Patton, S. *et al.*, *Biochim. Biophys. Acta*, **1241(3)**, 407-423 (1995).
5. Haltiwanger, R.S., and Lowe, J.B., *Annu. Rev. Biochem.*, **73**, 491-537 (2004).
6. Gendler, S.J., and Spicer, A.P., *Annu. Rev. Physiol.*, **57**, 607-634 (1995).
7. Naughton, J. *et al.*, *Gut Microbes*, **5(1)**, 48-52 (2014).
8. van Putten, J.P.M., and Strijbis, K., *J. Innate Immun.*, **9(3)**, 281-299 (2017).
9. Peng, W. *et al.*, *Expert Rev. Proteomics*, **15(12)**, 1007-1031 (2018).
10. Kuo, J.C.-H. *et al.*, *Nat. Phys.*, **14(7)**, 658-669 (2018).
11. Malaker, S.A. *et al.*, *Proc. Nat. Acad. Sci. USA*, **116(15)**, 7278-7287 (2019).

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