Recombinant *E. coli* Metalloprotease/StcE His-tag

Catalog Number: 11406-MP

DESCRIPTION

Source	*E. coli*-derived *e. coli* StcE protein Ala36-Lys898 with a N-terminal Met and 6-His tag Accession # O82882.2
N-terminal Sequence Analysis	Met & Arg415
Predicted Molecular Mass	97 kDa & 54 kDa

SPECIFICATIONS

SDS-PAGE	86-95 & 53-58 kDa, under reducing conditions.
Activity	Measured by its ability to cleave Recombinant CD45 Protein at specific O-glycan sites. One µg of Recombinant *E. coli* StcE will cleave >60% of Recombinant CD45 Protein with labeled O-glycan (Catalog # 1430-CD), as measured under the described conditions.
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>85%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Digestion Buffer: 50 mM Tris, 150 mM NaCl, pH 7.5
- Labeling Buffer: 50 mM HEPES, 10 mM $MnCl_2$, pH 7.0
- Recombinant *E. coli* StcE His-Tag (rE. StcE) (Catalog # 11406-MP)
- Recombinant Human CD45 (rhCD45) (Catalog # 1430-CD)
- Recombinant Human ST3GAL2 (rhST3GAL2) (Catalog # 7275-GT)
- Recombinant C. perfringens Neuraminidase (Catalog # 5080-NM)
- CMP-Cy3-Sialic Acid (Cy3-SA) (Catalog # ES402)
- 12% SDS-PAGE gel
- Gel loading dye
- Gel Imager with Cy3 fluorescent dye detection capability

Assay

1. Dilute rhCD45 to 200 µg/mL and rE. StcE to 100 µg/mL with Digestion Buffer.
2. Combine 10 µL of 200 µg/mL rhCD45 and 10 µL of 100 µg/mL rE StcE. For a control, combine 10 µL of Digestion Buffer and 10 µL of 200 µg/mL rhCD45.
3. Incubate at 37 °C for 2 hours.
4. Heat tubes for 2 minutes at 95 °C.
5. Create a labeling mixture containing 10 µg/mL rhST3GAL2, 2.5 µg/mL of Neuraminidase, and 10 µM of Cy3-SA with Labeling Buffer.
6. Add 20 µL of labeling mixture to each reaction and control.
7. Incubate at 37 °C for 1 hour.
8. Add Loading dye to each tube.
9. Load half the volume of each reaction and control onto a 12% SDS-PAGE gel. Let samples migrate at least 80% down the gel before stopping.
10. Acquire gel image and determine percent cleavage.

$$\% \text{ Cleavage} = \left[1 - \left(\frac{\text{Intensity of uncleaved}}{\text{Total Control Intensity}} \right) \right] \times 100$$

Final Assay Conditions

Per Reaction:

- rE. StcE: 1 µg
- rhCD45: 2 µg
- rhST3GAL2: 0.2 µg
- rCp Neuraminidase: 0.05 µg
- CMP-Cy3-Sialic Acid: 0.2 nmol

PREPARATION AND STORAGE

Shipping	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 6 months from date of receipt, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after opening.

DATA

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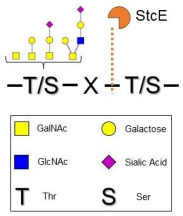
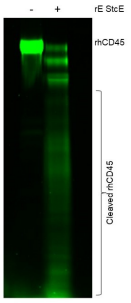
Enzyme Activity



Recombinant *E. coli* StcE His-tag Enzyme Activity Diagram. Recombinant *E. coli* StcE His-tag cleaves glycoproteins C-terminally to glycosylated Ser/Thr residues that are near the cleavage residue. Specificity for O-glycan complexity is broad. For simplicity not all O-glycans may be represented.

Enzyme Activity



Recombinant *E. coli* StcE His-tag Enzyme Activity Assay. Recombinant Human CD45 Protein, CF (Catalog # 1430-CD) was first cleaved using Recombinant *E. coli* StcE (Cat # 11406-MP). Following cleavage, Recombinant C. perfringens Neuraminidase Protein, CF (Catalog # 5080-NM) was used to remove intact Sialic Acid. O-glycans were then labeled using Recombinant Human ST3GAL2 Protein, CF (Catalog # 7275-GT) and CMP-Cy3-Sialic Acid (Catalog # ES402). Samples were then run on a SDS-PAGE gel and imaged using the green fluorescent channel.

BACKGROUND

Recombinant *E. coli* StcE (secreted protease of C1-esterase inhibitor) is a zinc-dependent, monomeric mucinase from Enterohaemorrhagic Escherichia coli (EHEC). EHEC is a major foodborne pathogen that must penetrate a thick mucus layer for effective infection. StcE has three distinct globular domains with non-catalytic globular domains at the N- and C-terminus and the catalytic metalloprotease domain in the middle. This open arrangement is thought to allow for additional regulatory functions and flexibility to recognize large O-glycosylated substrates (1, 2). StcE is secreted via the type II general secretory pathway during infection as a major virulence factor due to its protease-meditated activity towards heavily glycosylated proteins with high levels of O-linkages that play a host defensive role such as mucin 7 and glycoprotein 340, in addition to C1-esterase inhibitor, a regulator of inflammation pathways (2). StcE has also been implicated in cleavage of glycoproteins found on the surface of various cells such as intestinal epithelial cells and neutrophils, where it has been shown to lead to depression of neutrophil migration during infection (3, 4). StcE has been proposed as a treatment for cystic fibrosis as it is has been shown to loosen mucus in affected patients (1, 4). In addition, StcE has been proposed as a useful tool for the analysis of mucin-domain glycoproteins known to be important in several human diseases including cancer (5-8). StcE has a broad specificity from simple to complex O-glycans, with specificity for a discrete peptide- and glycan-based motif that allows its utilization in glycoproteomic mapping of mucin glycosites or enrichment of mucins (5-8). The activity of recombinant StcE is demonstrated in an electrophoretic gel mobility shift assay using enzymatically fluorophore labeled mucin-like glycoproteins.

References:

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