Recombinant *S. pyogenes* Endo-beta-N-acetylglucosaminidase S2/Endo S2 His-tag

Catalog Number: 10976-GH

DESCRIPTION

Source	*E. coli*-derived *s. pyogenes* Endo-beta-N-acetylglucosaminidase S2/Endo S2 protein Glu37-Asp843, with a N-terminal Met & 6-His tag Accession # ACI61688.1
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	92 kDa

SPECIFICATIONS

SDS-PAGE	86 - 93 kDa, under reducing conditions.
Activity	Measured by its ability to digest Cy5-Labeled Glycan G2 >50% of Cy5-Labeled Glycan G2 (0.2 pmol) is digested by 0.2 µg of rSp. Endo-S2, as measured under the described conditions.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>90%, 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

- Assay Buffer: 50 mM MES, pH 6.0
- Recombinant Sp. Endo-S2 (rSpEndo-S2) (Catalog # 10976-GH)
- Cy5-Labeled Glycan G2 (Cy5-G2) (Catalog # GL302)
- 15% SDS-PAGE gel and SDS-PAGE Reagents
- Reducing SDS-PAGE Sample Buffer
- FluorChem R System (by Protein Simple) or equivalent

Assay

1. Dilute rSpEndo-S2 to 20 ng/µL in Assay Buffer.
2. Dilute Cy5-G2 to 0.02 µM in Assay Buffer.
3. Prepare reaction by combine 10 µL of diluted rSpEndo-S2 and 10 µL of diluted Cy5-G2.
4. Prepare a negative control by combining 10 µL of diluted Cy5-G2 and 10 µL of Assay Buffer.
5. Incubate reaction(s) and negative control at 37 °C for 60 minutes.
6. Add 7 µL of reducing SDS-PAGE sample buffer to each reaction and negative control.
7. Load 13.5 µL of each sample and control per well on a 15% SDS-PAGE gel and run SDS-PAGE until the dye front has migrated more than two thirds of the way down the gel.
8. Acquire gel image with a FluorChem R System using the MultiFluor Red setting.
9. Analyze the amount (%) of Cy5-G2 digested by rSpEndo-S2 in each reaction lane.

Final Assay Conditions

Per Reaction:

- rSpEndo-S2: 0.20 µg
- Cy5-G2: 0.2 pmol

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Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449

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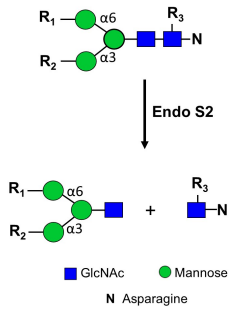
PREPARATION AND STORAGE

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.

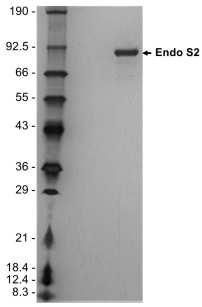
DATA

Enzyme Activity



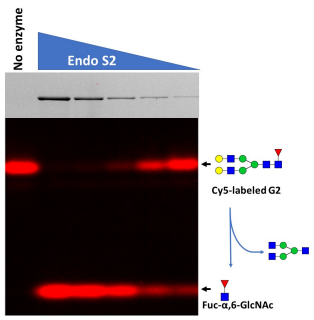
Recombinant Sp. Endo S2 (Endo S2) Enzyme Activity Diagram Endo S2 recognizes the conserved N-glycans on the Fc region of IgG by hydrolyzing the chitobiose core (at the β-1,4 linkage between the two N-acetylglucosamines) of the N-glycans. Endo S2 leaves one GlcNAc residue attached the the asparagine of the peptide backbone. R1 and R2 can be oligosaccharide extensions containing Gal, GlcNAc, and Sialic Acid. R3 can be unmodified or Core-6 Fucose.

SDS-PAGE



Recombinant S. pyogenes Endo S2 SDS-PAGE
1 µg/lane of rSp. Endo-S2 (Catalog # 10976-GH) was resolved with SDS-PAGE under reducing (R) conditions and visualized by silver staining, showing a band at 92 kDa.

Gel Supershift Assay



Fluorescent Gel Mobility Shift caused by rSp. Endo S2. Lane 1 contained substrate Cy5-labeled G2 Catalog # GL302. In the presence of Endo S2, the glycan was digested to products and the smaller product Fuc-α,6-GlcNAc is observed.

BACKGROUND

Streptococcus pyogenes is a leading Gram-positive bacterial pathogen that can abolish the effector functions of human immunoglobulin G (IgG) through deglycosylation (1). Upon infection, the pathogen secret two endoglycosidases, Endo S and Endo S2, that specifically deglycosylate the conserved N-glycans on the Fc region of IgG by hydrolyzing the chitobiose core (at the β-1,4 linkage between the two N-acetylglucosamines) of the N-glycans (2, 3). Endo S and S2 cleavage leave one GlcNAc residue remaining attached to the asparagine residue on the peptide backbone. The enzymes are highly specifc to native IgG molecules (3), suggesting that the local conformation of IgG is required for the enzymatic recognition. In comparison, PNGase F from *Flavobacterium meningosepticum* completely removes glycans from glycoproteins and is more active on denatured glycoproteins. Cleavage of the glycans from IgG antibodies by Endo S and S2 result in conformation change of the antibodies thereby dramatically diminish the binding affinity to their receptors (4) and abolish their opsonizing functions (5, 6). By using fluorophore labeled N-Glycans as substrates, we found that Endo S2 has similar activity to Endo S towards non-galactosylated IgG glycan species including oligomannose and hybrid glycans but shows much high activity on galactosylated and sialylated IgG glycan species. We also found that both enzymes are highly active on core-6 fucosylated IgG glycans but with no activities on those with bisecting GlcNAc.

References:

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