Recombinant *R. gnavus* α-L-Fucosidase His-tag

Catalog Number: 11385-GH

DESCRIPTION

Source	*E. coli*-derived alpha-L-Fucosidase protein Glu35-Gly583 with an N-terminal Met and 6-His tag Accession # WP_243035407.1
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	62 kDa

SPECIFICATIONS

SDS-PAGE	62-68 kDa, under reducing conditions
Activity	Measured by its ability to cleave α3 Fucose. Recombinant a-L-Fucosidase will cleave >50% Cy5-Fuc labeled Lewis X (Catalog # GL306), as measured under the described conditions.
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>95%, 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, 10 mM $CaCl_2$, 10 mM $MnCl_2$, pH 6.0
- Recombinant *R. gnavus* α-L-fucosidase (rRg α-L-fucosidase) (Catalog # 11385-GH)
- Cy5-Fuc labeled Lewis X (Cy5-Lewis X) (Catalog # GL306)
- 15% SDS-PAGE
- Gel loading dye
- Gel Imager with Cy5 fluorescent dye detection capability

Assay

1. Dilute rRg α-L-fucosidase to 5 µg/mL with Assay Buffer.
2. Dilute Cy5-Lewis X to 0.02 uM with Assay Buffer.
3. For reaction, combine 10 µL of rRg α-L-fucosidase and 10 µL of Cy5-Lewis X. For control, combine 10 µL of Assay Buffer and 10 µL of Cy5-Lewis X.
4. Incubate reaction and control at 37 °C for 30 minutes.
5. Add gel loading dye to each tube.
6. Load half the volume of each reaction and control onto a 15% SDS-PAGE gel. Let samples migrate at least 80% down the gel before stopping.
7. Acquire gel image and determine percent cleavage.

Final Assay Conditions

Per Reaction:

- rRg α-L-fucosidase: 0.05 µg
- Cy5-Lewis X: 0.2 pmol

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.

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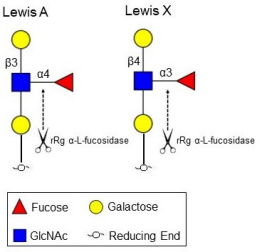
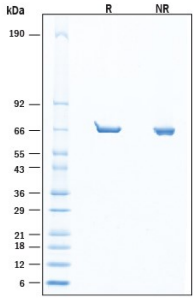
DATA

Enzyme Activity



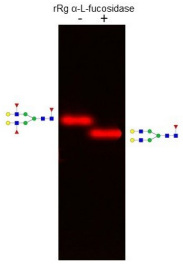
Recombinant R. gnavus α-L-Fucosidase His-tag Protein Enzyme Activity Diagram. Recombinant R. gnavus alpha-L-Fucosidase His-tag Protein, CF (Catalog # 11385-GH) has specificity for both Lewis A (α4) and Lewis X (α3) link fucose. Sialyation of Lewis A or Lewis X does not affect the substrate recognition of α-L-fucosidase.

SDS-PAGE



Recombinant R. gnavus α-L-Fucosidase His-tag Protein SDS-PAGE. 2 µg/lane of Recombinant R. gnavus α-L-Fucosidase His-tag Protein (Catalog # 11385-GH) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 62-68 kDa under reducing conditions.

Enzyme Activity



Recombinant R. gnavus α-L-Fucosidase His-tag Protein Enzyme Activity. Lane 1 contained fluorescent substrate glycan Cy5-Fuc labeled Lewis X. Following treatment with Recombinant R. gnavus alpha-L-Fucosidase His-tag Protein, CF (Catalog # 11385-GH) both α3 linked Fucose on GlcNAc were removed and a mobility shift was observed. SDS-PAGE gel was imaged using the red fluorescent channel.

BACKGROUND

Recombinant R. gnavus α-L-Fucosidase His-tag (α-L-Fuc) catalyzes the hydrolysis of terminal α-L-Fucose. α-L-Fuc is monomeric and consists of two distinct domains, an N-terminal catalytic domain comprising residues 46-366 and a C-terminal F5/8 Type C domain covering residues 385-526 (1). Residues 23-45 wrap around the C-terminal β-sandwich domain (1). R. gnavus is an early colonizer of the human gut but persists in healthy adults (2-3). An increasing number of studies are reporting a disproportionate representation of R. gnavus in diseases, such as inflammatory bowel disease (4). To access a source of nutrients, gut bacteria encode α-L-fucosidases that catalyze the hydrolysis of terminal α-L-fucosidic linkages. α-L-Fuc has the capacity to recognize fucosylated glycans and to hydrolyze both α1-3 (Lewis X) and α1–4 (Lewis A) fucosyl linkages although the preferred substrate is sialyated Lewis X epitope (1). This fucosidase specificity can potentially be exploited for use in human disease diagnostic assays, as a tool to identify N-glycan biomarkers of disease, and for glycoprofiling biopharmaceutical glycoproteins. The activity of α-L-Fuc is demonstrated in an electrophoretic gel mobility shift assay using a fluorophore-labeled glycan Cy5-Fuc labeled Lewis X as the substrate (5).

References:

1. Wu, H. *et al.* (2021) Cell. Mol. Life Sci. **78**:675.
2. Sagheddu, V. *et al.* (2016) Front. Pediatr. **4**:57.
3. Qin, J. *et al.* (2010) Nature **464**:59.
4. Hall, A.B. *et al.* (2017) Genome Med. **9**:103.
5. Wu, Z.L. *et al.* (2020) Glycobiology **30**:970.

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