Recombinant Human Plasma alpha-L-Fucosidase/FUCA2 His-tag

Catalog Number: 11405-GH

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived human Plasma alpha-L-Fucosidase/FUCA2 protein His29-Ile467 with an N-terminal 6-His tag Accession # Q9BTY2.2
N-terminal Sequence Analysis	His
Predicted Molecular Mass	52 kDa

SPECIFICATIONS

SDS-PAGE	52-58 kDa, under reducing conditions.
Activity	Measured by its ability to cleave a fluorogenic substrate 4-methylumbelliferyl-α-L-fucopyranoside. The specific activity is >550 pmol/min/µg, 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 Sodium Acetate, pH 4.5
- Recombinant Human Plasma alpha-L-Fucosidase/FUCA2 His-tag (rhFUCA2) (Catalog # 11405-GH)
- Substrate: 4-Methylumbelliferyl-alpha -L-fucopyranoside, 50 mM stock in DMSO
- Black 96 Well Plate
- Plate Reader with Fluorescence Read Capability

Assay

1. Dilute rhFUCA2 to 5 µg/mL in Assay Buffer.
2. Dilute Substrate to 1.6 mM in Assay Buffer.
3. Load into a plate 50 µL of 5 µg/mL rhFUCA2, and start the reaction by adding 50 µL of 1.6 mM Substrate. Include a Substrate Blank containing 50 µL of Assay Buffer and 50 µL of 1.6 mM Substrate.
4. Read plate at excitation and emission wavelengths of 365 nm and 445 nm, respectively, in kinetic mode for 5 minutes.
5. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (RFU/min) x Conversion Factor** (pmol/RFU)}}{\text{amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank
**Derived using calibration standard 4-Methylumbelliferone (4-MU)

Final Assay Conditions

Per Well:

- rhFUCA2: 0.25 µg
- Substrate: 0.8 mM

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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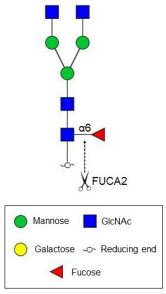
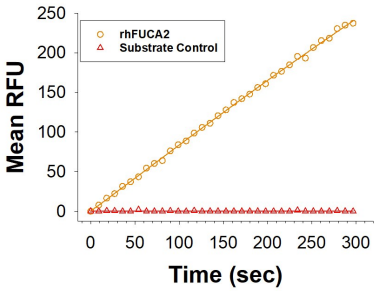
DATA

Enzyme Activity



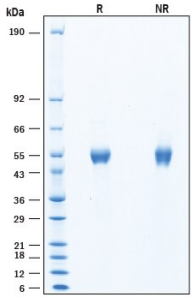
Recombinant Human Plasma alpha-L-Fucosidase/FUCA2 His-tag Protein, CF Enzyme Activity Diagram. Recombinant Human Plasma alpha-L-Fucosidase/FUCA2 His-tag Protein, CF (Catalog # 11405-GH) is involved in the hydrolysis of terminal L-fucose residues linked via α-1,2, α-1,3, α-1,4, or α-1,6. For simplicity only α-1,6 is displayed.

Enzyme Activity



Recombinant Human Plasma alpha-L-Fucosidase/FUCA2 His-tag Protein Enzyme Activity. Recombinant Human Plasma alpha-L-Fucosidase/FUCA2 His-tag Protein, CF (Catalog # 11405-GH) is measured by its ability to cleave a fluorogenic substrate 4-methylumbelliferyl-alpha -L-fucopyranoside.

SDS-PAGE



Recombinant Human Plasma alpha-L-Fucosidase/FUCA2 His-tag Protein SDS-PAGE. 2 µg/lane of Recombinant Human Plasma alpha-L-Fucosidase/FUCA2 His-tag Protein (Catalog # 11405-GH) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 52-58 kDa, under reducing conditions.

BACKGROUND

Plasma alpha-L-Fucosidase, also known as FucA2 (FUCA2), is an enzyme responsible for hydrolyzing alpha-L-fucose (1). FUCA2 and its isoform FUCA1 are human fucosidases of the glycoside hydrolase family 29A (2). Whereas FUCA2 is secreted and localized to plasma, FUCA1 is expressed in cellular tissues in the lysosome (3). The mature FUCA2 enzyme exists in the native state as a homotetramer and contains a highly conserved eukaryotic alpha fucosidase catalytic N-terminal barrel structure. In comparison to FUCA1, FUCA2 may not contain a catalytic residue in the sixth beta strand of the barrel structure which may suggest differing substrate specificity or catalytic mechanism (2). FUCA2 has specificity for fucose linked via α-1,2, α-1,3, α-1,4, or α-1,6 to the reducing end of glycans (4). As fucosylated glycoconjugates play numerous roles in biological events such as development, apoptosis, and the immune response, fucosylated glycoconjugates are involved in the pathology of inflammation, cancer, and cystic fibrosis (5-8). FUCA2 has been implicated to play a role in several human diseases; FUCA2 was found to be upregulated in 24 tumor types and was associated with negative outcomes for several of the cancer types (9). In addition, FUCA2 was found to play a major role in bacterial adhesion in H. pylori-induced diseases like gastric cancer and duodenal ulcers (10). Consequently, FUCA2 may be of use in both diagnostic and therapeutic research.

References:

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