

DESCRIPTION

Source Mouse myeloma cell line, NS0-derived
Gln32-Lys466 with an N-terminal CD33 signal sequence and a C-terminal 6-His tag
Accession # P04066

N-terminal Sequence Analysis Gln32

Predicted Molecular Mass 51 kDa

SPECIFICATIONS

SDS-PAGE 55-60 kDa, reducing conditions

Activity Measured by its ability to cleave a fluorogenic substrate 4-methylumbelliferyl-α-L-fucopyranoside. The specific activity is >4,800 pmol/min/μg, as measured under the described conditions. See Activity Assay Protocol on www.RnDSystems.com.

Endotoxin Level <1.0 EU per 1 μg of the protein by the LAL method.

Purity >95%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 μg per lane.

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, 5 mM MgCl₂, pH 4.5
- Recombinant Human Tissue α-L-Fucosidase/FUCA1 (rhFUCA1) (Catalog # 7039-GH)
- Substrate: 4-Methylumbelliferyl-α-L-fucopyranoside (Research Products International Corp, Catalog # M65200), 50 mM stock in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhFUCA1 to 2 ng/μL in Assay Buffer.
2. Dilute Substrate to 1.6 mM in Assay Buffer.
3. Load into a plate 50 μL of 2 ng/μL rhFUCA1, and start the reaction by adding 50 μL of 1.6 mM Substrate. For Substrate Blanks, load 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:

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

*Adjusted for Substrate Blank

**Derived using calibration standard 4-Methylumbelliferone (4-MU) (Sigma, Catalog # M1381).

Final Assay Conditions Per Well:

- rhFUCA1: 0.100 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.

BACKGROUND

Fucosylated glycoconjugates play numerous roles in biological processes, such as development, apoptosis and the immune responses, and are involved in the pathology of inflammation, cancer and cystic fibrosis (1, 2, 3, 4). Tissue α-L-Fucosidase, also known as FUCA1, is a lysosomal enzyme responsible for hydrolyzing α-L-fucose moieties from glycolipids and oligosaccharides (5). The mature enzyme in the liver has a molecular weight of 200 kDa and exists in the native state as a tetramer (6). Defects in FUCA1 are the cause of fucosidosis (7, 8, 9), an autosomal recessive lysosomal storage disease characterized by the accumulation of fucose-containing glycolipids and glycoproteins in various tissues. Serum α-L-fucosidase has been identified as a useful marker for oral cancer (10).

References:

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