Recombinant *T. maritima* α-L-Fucosidase  
Catalog Number: 6556-GH

**DESCRIPTION**

Source  
*E. coli*-derived Ile2-Glu449, with an N-terminal Met and 6-His tag  
Accession # NP_228118

N-terminal Sequence Analysis  
Met

Predicted Molecular Mass  
53 kDa

**SPECIFICATIONS**

SDS-PAGE  
43-50 kDa, reducing conditions

Activity  
Measured by its ability to cleave a fluorogenic substrate 4-methylumbelliferyl-α-L-fucopyranoside. The specific activity is >1,600 pmol/min/µg, as measured under the described conditions.

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 MES, 200 mM NaCl, pH 5.5
- Recombinant *T. maritima* α-L-Fucosidase (*r*.*T. maritima* α-L-Fucosidase) (Catalog # 6556-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 *r*T. *maritima* α-L-Fucosidase to 2 ng/µL in Assay Buffer.
2. Dilute Substrate to 400 µM in Assay Buffer.
3. Load into a plate 50 µL of 2 ng/µL *r*T. *maritima* α-L-Fucosidase, and start the reaction by adding 50 µL of 400 µM Substrate. For Substrate Blanks, load 50 µL of Assay Buffer and 50 µL of 400 µM 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:

   \[
   \text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{\text{max}}^\ast (RFU/\text{min}) \times \text{Conversion Factor}^\ast (\text{pmol/RFU})}{\text{amount of enzyme (µg)}}
   \]

   *Adjusted for Substrate Blank  
   **Derived using calibration standard 4-Methylumbelliferone (Sigma, Catalog # M1381).

**Final Assay Conditions**

**Per Well:**

- *T. maritima* α-L-Fucosidase: 0.100 µg
- Substrate: 200 µM

**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 events, including development and apoptosis (1, 2), and are involved in the pathology of inflammation, cancer, and cystic fibrosis (3, 4). Fucosidases are generally used for studying fucosylated glycans. With a *K*<sub>cat</sub>/*K*<sub>m</sub> value of 160,000 M<sup>-1</sup>s<sup>-1</sup>, the α-L-fucosidases of *Thermotoga maritima* efficiently hydrolyzes 4-nitrophenyl fucose (5). The enzyme is the closest bacterial relative of mammalian fucosidase with 38% identity to its human homologue. It is thought to remove α-1,2- and α-1,4-linked fucosyl side chains from algal fucoidan (5), while the activity on α-1,3- and α-1,6- linked fucose has not been tested. The enzyme assembles as a hexamer and displays a two-domain fold, with Asp<sup>224</sup> and Glu<sup>261</sup> being critical for enzyme activity (6).

**References:**