Recombinant Human Gastric Lipase/LIPF HA-tag His-tag

Catalog Number: 11772-GL

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived human Gastric Lipase protein Leu20-Lys398 with N-terminal HA (YPYDPDYA) and 6-His tags Accession # P07098.1
N-terminal Sequence Analysis	Tyr
Predicted Molecular Mass	45 kDa

SPECIFICATIONS

SDS-PAGE	49-54 kDa, under reducing conditions
Activity	Measured by its ability to cleave a fluorogenic substrate, 4-Methylumbelliferyl oleate (4-MUO). The specific activity is >4500 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, NaCl and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 500 mM NaCl, 1 mM $CaCl_2$, 1% Triton X-100, pH 7.5
- Recombinant Human Gastric Lipase/LIPF (rhLIPF) (Catalog # 11772-GL)
- Substrate: 4-Methylumbelliferyl oleate, 100 mM stock in DMSO
- Black 96-well Plate
- Plate Reader with Fluorescence Read Capability

Assay

1. Dilute rhLIPF to 0.05 µg/mL in Assay Buffer.
2. Dilute Substrate to 1.2 mM in Assay Buffer.
3. Load into a plate 50 µL of 0.05 µg/mL rhLIPF and start the reaction by adding 50 µL of 1.2 mM Substrate. Include a Substrate Blank containing 50 µL of Assay Buffer and 50 µL of 1.2 mM Substrate.
4. Read at excitation and emission wavelengths of 365 nm and 445 nm (top read), 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 a fluorescent standard 4-Methylumbelliferone

Final Assay Conditions

Per Well:
rhLIPF: 0.0025 µg
Substrate: 600 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.

DATA

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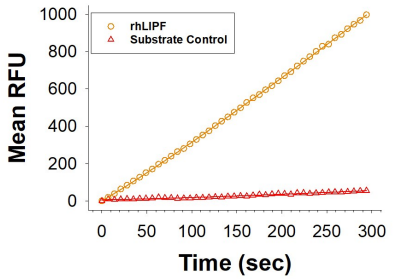
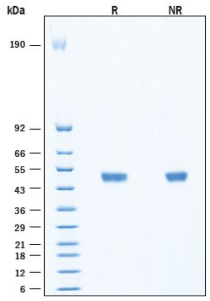
Enzyme Activity



Recombinant Human Gastric Lipase/LIPF HA-tag His-tag Enzyme Activity. Recombinant Human Gastric Lipase/LIPF HA-tag His-tag (Catalog # 11772-GL) is measured by its ability to cleave a fluorogenic substrate, 4-Methylumbelliferyl oleate (4-MUO).

SDS-PAGE



Recombinant Human Gastric Lipase/LIPF HA-tag His-tag SDS-PAGE. 2 µg/lane of Recombinant Human Gastric Lipase/LIPF HA-tag His-tag (Catalog # 11772-GL) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands 49-54 kDa, under reducing conditions.

BACKGROUND

Recombinant human Gastric triacylglycerol lipase or gastric lipase (GL), from the gene LIPF, is a 379 residue mature, glycosylated, secreted serine hydrolase of a family of three mammalian acid lipases. Mature GL/LIPF contains a globular core domain typical of a/b hydrolase-fold family members and an extrusion domain that covers the core domain and functions like a lid. Beneath the lid region, the core domain contains the catalytic triad surrounded by a hydrophobic surface that promotes lipid substrate binding (1). The lid domain inhibits binding of substrate and requires conformational change for adsorption at the interface of emulsified triacylglycerol in the presence of phospholipids and proteins to facilitate activity (2). GL/LIPF is secreted by the mucosal cells of the stomach where it initiates the digestion of dietary triglyceride fat through its preference for hydrolysis at the ester bond at carbon 3 (1, 3, 4). Although GL/LIPF contributes to a lower effect in the hydrolysis of dietary fats in the stomach than pancreatic lipase (PL) in the small intestine, gastric lipolysis contributes significantly to overall lipolysis and has an important role in promoting the action of pancreatic lipolytic enzymes through its actions (2,5-8). Therefore, inhibition of GL/LIPF along with PL, such as through the action of Orlistat, has been identified as a target for the prevention and treatment of obesity-related disorders such as diabetes, dyslipidemia, nonalcoholic fatty liver disease and cardiovascular disease (8-12). With a few available lipase inhibitors already in use in the clinic to manage obesity and its related disorders, significant investigation and development of additional or alternative inhibitors with fewer side effects is underway (3, 8). Recombinant GL/LIPF is recognized as a good candidate for enzyme replacement therapies for pancreatic enzyme insufficiency observed in chronic pancreatitis and cystic fibrosis (CF) due to its unique properties; unlike PL, no cofactor is required for GL/LIPF activity, GL/LIPF retains activity at low pH and in the presence of proteases, and GL/LIPF is not inhibited by bile salts (2, 7). In cases of pancreatic exocrine insufficiency, compensation for the loss of hepatic PL by GL has been previously noted as a common compensation mechanism that occurs naturally (7). Processing by gastric lipase in the stomach can also impact the integrity of oral drug delivery systems with premature drug release into the gastrointestinal environment and exposure of the drug to alteration from proteases (2, 13, 14) such that gastric digestion models (6, 13) have been developed to better understand potential impacts to oral pharmaceutical drug delivery systems (2, 13, 14).

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