Recombinant Human PPT1 HA-tag His-tag

Catalog Number: 11661-PT

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived human PPT1 protein Asp28-Gly306 with N-terminal HA (YPYDVPDYA) and 6-His tags Accession # P50897.1
N-terminal Sequence Analysis	Tyr
Predicted Molecular Mass	33 kDa

SPECIFICATIONS

SDS-PAGE	33-40 kDa, under reducing conditions
Activity	Measured by its ability to cleave the palmitoyl thioester linkage in 4-methylumbelliferyl-6-thio-palmitate-beta-D-glucopyranoside in a coupled reaction. The specific activity is >250 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 Citrate, 0.4% (v/v) Triton X-100, pH 4.0
- Stop Solution: 0.5 M Glycine, 0.3 M NaOH (~pH 10.0)
- Coupling Enzyme: Recombinant Human Cytosolic beta-Glucosidase/GBA3 (rhGBA3) (Catalog # 5969-GH)
- Recombinant Human PPT1 HA-tag His-tag (rhPPT1) (Catalog # 11661-PT)
- Substrate: 4-Methylumbelliferyl 6-thio-Palmitate-β-D-Glucopyranoside, 10 mM in DMSO
- Black 96-Well Plate
- Plate Reader with Fluorescence Read Capability

Assay

1. Dilute rhPPT1 to 1 µg/mL in Assay Buffer.
2. Create a master mix containing 40 µg/mL rhGBA3 and 1 mM Substrate in Assay Buffer.
3. Load 25 µL of 1 µg/mL rhPPT1 into wells of a plate and start the reactions by adding 25 µL of master mix. Include a Substrate Blank containing 25 µL of Assay Buffer and 25 µL of master mix.
4. Seal plate and incubate on the bench top for 20 minutes.
5. After incubation, stop the reactions by adding 50 µL of Stop Solution to each well.
6. Read at excitation and emission wavelengths of 365 nm and 445 nm (top read) in endpoint mode.
7. Calculate specific activity:

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

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

Final Assay Conditions

Per Well:

- rhPPT1: 0.025 µg
- rhGBA3: 1 µg
- Substrate: 0.5 mM

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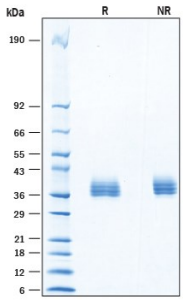
PREPARATION AND STORAGE

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

SDS-PAGE



Recombinant Human PPT1 HA-tag His-tag Protein SDS-PAGE. 2 µg/lane of Recombinant Human PPT1 HA-tag His-tag Protein (Catalog # 11661-PT) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 33-40 kDa, under reducing conditions.

BACKGROUND

Recombinant human palmitoyl-protein thioesterase 1 (PPT1), also known as CLN1, is one of two PPT lysosomal thioesterase proteins that catalyze the hydrolysis of long-chain fatty acids (1, 2). While the two PPT enzymes share 18% identity and some overlap in functionality, their specificities differ; PPT1 is the key enzyme responsible for catalyzing the removal of palmitate from S-palmitoylated proteins to facilitate their degradation and clearance from the lysosome (2-4). PPT1 is a glycosylated, monomeric protein that contains a signal peptide, a canonical α/β-hydrolase fold, a catalytic triad, and a fatty-acid hydrophobic groove binding site for palmitate (4). Mutations in PPT1 resulting in defects in activity lead to accumulation of lipid-modified proteins and cause fatal neurodegenerative lysosomal storage disorders known as neuronal ceroid lipofuscinoses (NCL) or Batten disease (4-6). As PPT1 plays a regulatory role in the autophagy-lysosome pathway, it is also a target for several types of cancer including hepatic, melanoma, and oral squamous cell carcinoma (7-10). Pharmacological methods for targeting of PPT1 via gene therapy, enzyme replacement therapy, or enzymatic-related inhibition are under investigation for the treatment of both NCLs and cancer (7, 8,11-13).

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