

APH975Hu01 100µg

Active Glycosylphosphatidylinositol Specific Phospholipase D1 (GPLD1)

Organism Species: *Homo sapiens* (Human)

Instruction manual

FOR RESEARCH USE ONLY

NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Prokaryotic expression.

Host: *E. coli*

Residues: Met496~Asp840

Tags: N-terminal His-tag

Purity: >90%

Endotoxin Level: <1.0EU per 1µg (determined by the LAL method).

Buffer Formulation: PBS, pH7.4, containing 0.01% Sarcosyl, 5%Trehalose .

Original Concentration: 200µg/mL

Applications: Activity Assays.

(May be suitable for use in other assays to be determined by the end user.)

Predicted isoelectric point: 6.9

Predicted Molecular Mass: 40.6kDa

Accurate Molecular Mass: 41kDa as determined by SDS-PAGE reducing conditions.

[USAGE]

Reconstitute in 10mM PBS (pH7.4) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.

Store at 2-8°C for one month.

Aliquot and store at -80°C for 12 months.

Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[SEQUENCE]

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                                                    MSSSP
NITISCQDIY CNLGWTLAA DVNGDSEPD L VIGSPFAPGG GKQKGIVAAF
YSGPSLSDKE KLNVEAANWT VRGEEDFSWF GYSLHGVTVD NRTLLLVGSP
TWKNASRLGH LLHIRDEKKS LGRVYGYFPP NGQSWFTISG DKAMGKLGTS
LSSGHVLMNG TLKQVLLVGA PTYDDVSKVA FLTVTLHQGG ATRMYALTSD
AQPLLLSTFS GDRRFSRFGG VLHLSDLDDD GLDEIIMAAP LRIADVTSG L
IGGEDGRVYV YNGKETTLGD MTGKCKSWIT PCPEEKAQYV LISPEASSRF
GSSLITVRSK AKNQVVIAAG RSSLGARLSG ALHVYSLGSD
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[ACTIVITY]

Glycosylphosphatidylinositol Specific Phospholipase D1 (GPLD1) is a secreted enzyme that cleaves glycosylphosphatidylinositol (GPI) anchors, releasing GPI-anchored proteins from cell membranes into circulation. Primarily synthesized in the liver, it plays key roles in lipid metabolism, inflammation, and neurogenesis. GPLD1's activity modulates the bioavailability of important GPI-anchored proteins like alkaline phosphatase and folate receptors. Studies link elevated GPLD1 levels to metabolic disorders and aging-related cognitive decline, while its neuroprotective effects are being investigated. The enzyme's structure contains catalytic domains essential for hydrolyzing GPI linkages, making it a potential therapeutic target for metabolic and neurological diseases. Besides, Alkaline Phosphatase, Tissue-nonspecific (ALPL) has been identified as an interactor of GPLD1, thus a functional binding ELISA assay was conducted to detect the interaction of recombinant human GPLD1 and recombinant human ALPL. Briefly, GPLD1 was diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate samples of 100 µl were then transferred to ALPL-coated microtiter wells and incubated for 1h

at 37 °C. Wells were washed with PBST and incubated for 1h with anti-GPLD1 pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody for 1h at 37 °C, wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37 °C. Finally, add 50 μ L stop solution to the wells and read at 450/630nm immediately. The binding activity of recombinant human GPLD1 and recombinant human ALPL was shown in Figure 1, the EC₅₀ for this effect is 0.042 μ g/mL.

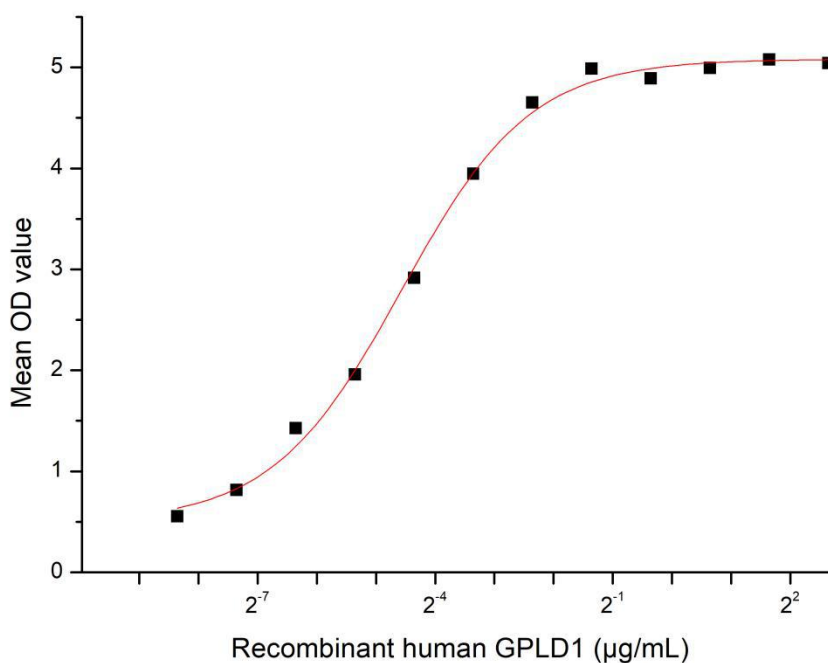


Figure 1. The binding activity of recombinant human GPLD1 and recombinant human ALPL

ATGTCCTCTGCCCCATCACTACCACTTTCTGCGCGAGCACTACTGTGTACTTGGCGTGGCTCTCTTGGCTGCAGATGTGAATGGAGCAGTGAACCCGCTCTGGTGCTGGCTGCCCTTTTGGACCGAGTGGAGGGAAGCGAGAGGAAATGTGGCTGGCTTTTATCTTGGCCCCGCTTGAGCGACGAGAAAACTGAAGCTGGAGGACGCCATCTGGCGGGTGAAGGG
MSSSPNITITISCDIYCNLGLWTLAADVNGDSEPDLVIGSPFAPGGGQKGIVAAAFYSGPSLSDKEKLNVEAANWTVRG

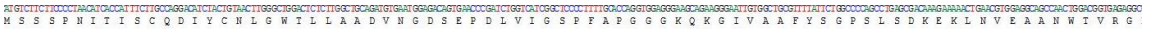


Figure 2. Gene Sequencing (extract)

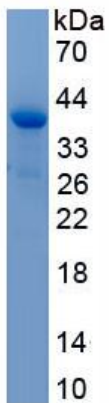


Figure 3. SDS-PAGE

Sample: Active recombinant GPLD1, Human

[IMPORTANT NOTE]

The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.