

APD761Hu01 100µg

Active Glutamine synthetase (GS)

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: Met1~Asn373

Tags: N-terminal His-tag

Purity: >95%

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: Cell culture; Activity Assays.

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

Predicted isoelectric point: 6.5

Predicted Molecular Mass: 43.6kDa

Accurate Molecular Mass: 45kDa 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.

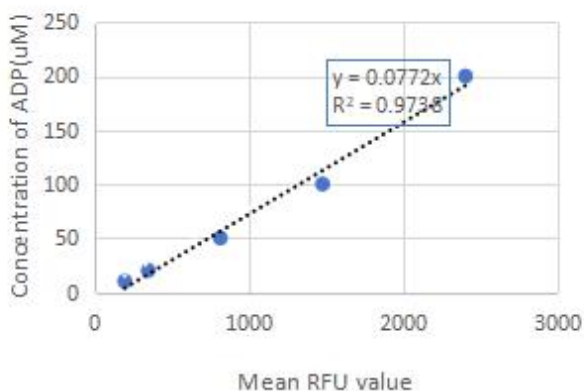
[SEQUENCE]

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MTTSASSHLN KGIKQVYMSL PQGEKVQAMY IWIDGTGEGE RCKTRTLDSE PKCVEELPEW
NFDGSSTLQS EGSNSDMYLV PAAMFRDPFR KDPNKLVLCE VFKNRRPAE TNLRHCTCKRI
MDMVSNQHPW FGMEQEYTLN GTDGHFPGWP SNGFPGPQGP YYCGVGADRA YGRDIVEAHY
RACLYAGVKI AGTNAEVMPA QWEFQIGPCE GISMGDHLWV ARFILHRVCE DFGVIATFDP
KPIPGNWNKA GCHTNFSTKA MREENGLKYI EEAIEKLSKR HQYHIRAYDP KGGLDNARRL
TGFHETSNIN DFSAGVANRS ASIRIPRTVG QEKKGYPEDR RPSANCDPFS VTEALIRTCL
LNETGDEPFQ YKN
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[ACTIVITY]

Glutamine synthetase (GS) is an evolutionarily conserved, ubiquitous enzyme. It catalyzes ATP-dependent glutamate-ammonia condensation to form glutamine, a key step in nitrogen metabolism, ammonia detoxification, and nitrogen storage. With oligomeric structures and species-specific isoforms, it's vital for animal liver/brain function, plant nitrogen assimilation, and microbial survival in nitrogen-poor environments. GS catalyzes the reaction of L-glutamate, NH_4^+ , and ATP to produce L-glutamine, ADP, and inorganic phosphate (Pi). The activity of recombinant human GS is measured by detecting the concentration of ADP generated in the reaction system using an ADP detection kit. GS perform gradient dilution to obtain concentrations of 100ug/ml, 50ug/ml, 25ug/ml, 12.5ug/ml, 6.25ug/ml, 3.13ug/ml, 1.56ug/ml, 0ug/ml. Prepare a 200 uL enzymatic reaction system according to the following components: 20uL 10mM MgCl_2 , 40uL 20mM L-glutamate, 20uL 10mM NH_4Cl , 50uL Enzyme sample. Add ATP to the system at a final concentration of 200uM (4uL of 100mM stock solution), mix rapidly, immediately place in a 37° C incubator, and react precisely for 30 minutes. Detect the fluorescence intensity of each well. Take the ADP standard from the ADP detection kit and perform gradient dilution to obtain concentrations of 200uM,

100uM, 50uM, 20uM, 10uM, 0uM. Plot a standard curve with the concentration of ADP standard (mM) as the x-axis and the corresponding detection value (e.g., absorbance or fluorescence intensity) as the y-axis. Using this standard curve, we calculate the corresponding concentration and subsequently determine the specific enzyme activity. The specific activity of recombinant human GS is > 100 pmol/min/ug.



RFU (560/590)	ADP (product) uM
192	10
344.2	20
812.6	50
1476.6	100
2404.6	200

Figure 1. The standard curve of ADP

Specific Activity (pmol/min/μg) =

$$\frac{\text{Adjusted Vmax} * (\text{RFU/min}) \times \text{Conversion Factor} ** (\text{pmol/RFU})}{\text{amount of enzyme (ug)}}$$

*Adjusted for Substrate Blank

**Derived using calibration standard ADP

[IDENTIFICATION]

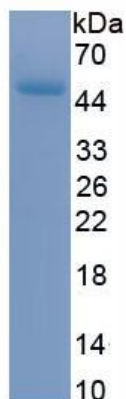


Figure 2. SDS-PAGE

Sample: Active recombinant GS, 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.