Recombinant Guinea Pig Asparaginase His-tag

Catalog Number: 10238-AS

DESCRIPTION

Source	*E. coli*-derived guinea pig Asparaginase protein Ala2-Ile565 Accession # XP_003463186 with an N-terminal Met and 6-His tag
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	62 kDa

SPECIFICATIONS

SDS-PAGE	57 kDa, under reducing conditions
Activity	Measured by its ability to convert asparagine to aspartic acid. The specific activity is >2700 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 visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in Tris, NaCl and TCEP. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 25 mM Tris, pH 7.5
- Recombinant Guinea Pig Asparaginase His-tag (rgpASPG) (Catalog # 10238-AS)
- Substrate: L-Asparagine (Sigma, Catalog # A0884), 100 mM stock in Assay Buffer
- α-Ketoglutaric Acid (Sigma, Catalog # K2010), 1 M stock in deionized water
- β-Nicotinamide adenine dinucleotide, reduced disodium salt hydrate (NADH) (Sigma, Catalog #
- N8129), 20 mM stock in 0.1 M Sodium Borate, pH 9.0
- Glutamic-Oxalacetic Transaminase (Sigma, Catalog # G2751)
- Malic Dehydrogenase (Sigma, Catalog # M2634)
- 96-well Clear Plate (Catalog # DY990)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Prepare a Reaction Mixture containing 800 µM NADH, 800 µM α-Ketoglutaric Acid, 8 Unit/mL Glutamic-Oxalacetic Transaminase and 4 unit/mL Malic Dehydrogenase in Assay Buffer.
2. Dilute L-Asparagine to 20 mM in Assay Buffer.
3. Dilute rgpASPG to 8 ng/µL in Assay Buffer.
4. Load in plate, 25 µL of 8 ng/µL rgpASPG and add 25 µL of Reaction Mixture to all wells. Include a Substrate Blank containing 25 µL Assay Buffer and 25 µL of Reaction Mixture.
5. Start the reaction by adding 50 µL of 20 mM L-Asparagine to all wells.
6. Read plate in kinetic mode for 5 minutes at an absorbance of 340 nm.
7. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (OD/min) x well volume (L) x } 10^{12} \text{ pmol/mol x -1}}{\text{ext. coeff** } (M^{-1}cm^{-1}) \text{ x path corr.*** (cm) x amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Using the extinction coefficient 6220 $M^{-1}cm^{-1}$

***Using the path correction 0.32 cm

Note: the output of many spectrophotometers is in mOD

Final Assay Conditions

Per Well:

- rgpASPG: 0.2 µg
- L-Asparagine: 10 mM
- NADH: 200 µM
- α-Ketoglutaric Acid: 200 µM
- Glutamic-Oxalacetic Transaminase: 2 Units
- Malic Dehydrogenase: 1 Unit

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

bio-techne®
Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449

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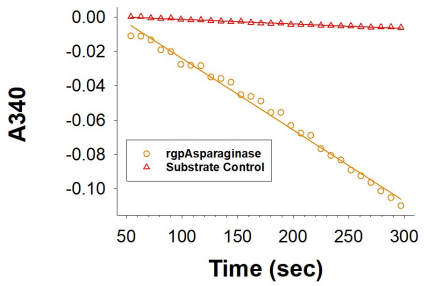
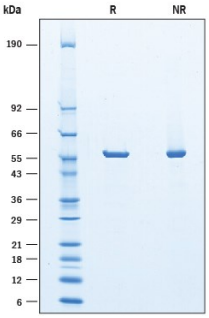
Binding Activity



Recombinant Guinea Pig ASPG His-tag (Catalog # 10238-AS) is measured by its ability to convert asparagine to aspartic acid.

SDS-PAGE



2 µg/lane of Recombinant Guinea Pig Asparaginase His-tag was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing a band under reducing conditions at 57 kDa.

BACKGROUND

L-Asparaginases catalyze the conversion of L-asparagine (Asn) to L-aspartic acid (Asp) and play important roles in amino acid metabolism of significance in medical, antimicrobial, and food industry applications (1). Guinea pig ASPG (gpASPG), also known as ASNase1, is a tetramer composed of a dimer of dimers. Each protomer contains a N-terminal domain with the active site and a C-terminal domain that can act as a lid when substrate is bound (2). gpASPG was identified as an L-asparaginase capable of inhibiting tumor growth (3, 4). It was observed that unlike most normal cells, in acute lymphatic leukemia (ALL) there is little or no detectable asparagine synthetase making cancer cells specifically sensitive to extracellular asparagine depletion with asparaginase treatment (5-7). Although bacterial asparaginases are currently available as therapeutics in ALL (8), treatment using bacterial origin asparaginase leads to immunogenic intolerance and clearance issues from the blood (9). Additionally, the bacterial asparaginases also show an unnecessary side L-glutaminase activity (10). For these reasons, a human-like enzyme would be preferred for therapeutic use (9, 11) but must have efficient hydrolysis of extracellular Asn with a Km value in the low micromolar range (2). Human ASPG is unable to match the required efficiency for therapeutic use (2, 12, 13), but gpASPG is homologous and has similar kinetic efficiency to the bacterial enzymes currently being used as therapeutics (2, 14). gpASPG may consequently make an appropriate mammalian-source therapeutic for replacement. There is significant focus on asparaginases given the broad beneficial applications for these enzymes.

References:

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