Recombinant Human Asparagine Synthetase/ASNS His-tag

Catalog Number: 10193-AS

DESCRIPTION

Source	Human embryonic kidney cell, HEK293-derived human Asparagine Synthetase/ASNS protein Cys2-Ala561 Accession # P08243-1 with C-terminal 6-His tag
N-terminal Sequence Analysis	Cys
Predicted Molecular Mass	65 kDa

SPECIFICATIONS

SDS-PAGE	58-65 kDa, under reducing conditions
Activity	Measured by its ability to produce diphosphate during the conversion of aspartate and glutamine to asparagine and glutamate. The specific activity is >100 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, TCEP and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 100 mM Tris, 100 mM NH_4Cl, 10 mM $MgCl_2$, pH 8.0
- Recombinant Human Asparagine Synthetase/ASNS (rhASNS) (Catalog # 10193-AS)
- Recombinant Yeast Inorganic Pyrophosphatase/PPA1 (ryPPA1) (Catalog # 8088-PP)
- L-Aspartic Acid (Sigma, Catalog # A7219), 200 mM stock in deionized water
- Adenosine triphosphate (ATP) (Sigma, Catalog # A7699), 400 mM stock in deionized water
- L-Glutamine (Sigma, Catalog # G8540), 200 mM stock in deionized water
- Malachite Green Phosphate Detection Kit (Catalog # DY996)
- 96-well Clear Plate (Catalog # DY990)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute 1 M Phosphate Standard by adding 10 µL of the 1 M Phosphate Standard to 990 µL of deionized water for a 10 mM stock. Continue by adding 10 µL of the 10 mM Phosphate stock to 990 µL of Assay Buffer for a 100 µM stock. This is the first point of the standard curve.
2. Continue standard curve by performing six one-half serial dilutions of the 100 µM Phosphate stock using Assay Buffer. The standard curve has a range of 0.078 to 5 nmol per well.
3. Prepare reaction mixture containing 2 mM ATP, 8 mM L-Aspartic, 40 mM L-Glutamine and 8 µg/mL ryPPA1 in Assay Buffer.
4. Dilute rhASNS to 16 µg/mL in Assay Buffer.
5. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of Assay Buffer.
6. Load 25 µL of the 16 µg/mL rhASNS into empty wells of the same plate as the curve. Include a Control containing 25 µL of Assay Buffer.
7. Add 25 µL of the reaction mixture to all wells, excluding the standard curve.
8. Seal plate and incubate at room temperature for 10 minutes.
9. Add 30 µL of the Malachite Green Reagent A to all wells. Mix briefly.
10. Add 100 µL of deionized water to all wells. Mix briefly.
11. Add 30 µL of the Malachite Green Reagent B to all wells. Mix and incubate sealed plate for 20 minutes at room temperature.
12. Read plate at 620 nm (absorbance) in endpoint mode.
13. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Phosphate released* (nmol) x (1000 pmol/nmol)}}{\text{Incubation time (min) x amount of enzyme (µg) x 2}}$$

*Derived from the phosphate standard curve using linear or 4-parameter fitting and adjusted for Control.

Final Assay Conditions

Per Reaction:

- rhASNS: 0.4 µg
- ryPPA1: 0.2 µg
- ATP: 1 mM
- L-Aspartic Acid: 4 mM
- L-Glutamine: 20 mM

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.

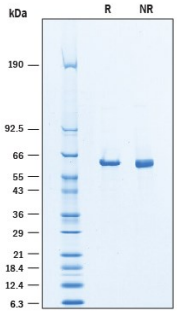
bio-techne®
Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449

Recombinant Human Asparagine Synthetase/ASNS His-tag

Catalog Number: 10193-AS

DATA

SDS-PAGE



2 µg/lane of Recombinant Human Asparagine Synthetase/ASNS His-tag was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® blue staining, showing a band at ~62 kDa under reducing conditions.

BACKGROUND

Asparagine Synthetase (ASNS) represents a single mammalian-expressed enzyme that catalyzes the synthesis of asparagine and glutamate from aspartate and glutamine in an ATP-dependent amidotransferase reaction (1). Although the name focuses attention on its function in asparagine synthesis, the reaction catalyzed by ASNS also impacts glutamine, aspartate and glutamate homeostasis. Human ASNS is a class II or N-terminal nucleophile glutamine amidotransferase (2) and is a cytosolic, 65 kDa ATP-dependent homodimer where each monomer is composed of two functional domains based on modeling: an N-terminal domain that contains the glutamine-binding pocket and the C-terminal domain that contains the ATP-binding site (3). Although ubiquitously expressed at low levels in many organs, expression is particularly high in the pancreas (4). Several mutations in the ASNS gene which are suspected to result in decreased activity cause Asparagine Synthetase Deficiency (ASD) (5). ASD has been characterized as a neurological disorder with severe impacts on psychomotor development and mortality at a young age supporting involvement of asparagine in neural development (6). As deficiency of ASNS leads to extracellular asparagine dependence, the brain is highly susceptible to deficiency due to the blood brain barrier limitation of available amino acids (7). Interest in targeting asparagine metabolism in cancer is based on the observation that unlike most normal cells, in acute lymphatic leukemia (ALL) there is little or no detectable ASNS nor upregulation during substrate deprivation in leukemic lymphoblasts making the transformed cells specifically sensitive to extracellular asparagine depletion (5, 8-10). Lower ASNS levels correlates with reduced proliferative capacity while subsequent supplementation of asparagine led to increased cell survival (11). Treatment with bacterial asparaginase as a component in combinatorial chemotherapy depletes plasma asparagine levels and results in starving leukemia cells in ALL to prevent growth (12) and can be applied more broadly to solid tumors where ASNS expression levels correlate with asparaginase sensitivity (10, 13-15).

References:

1. Richards, N. G. J. and Schuster, S.M. (1998) Adv. Enzymol. **72**:145.
2. Zalkin, H. and J. L. Smith (1998) Adv. Enzymol. Relat. Areas Mol. Biol. **72**:87.
3. Larsen, T. M. *et al.* (1999) Biochemistry. **38**:16146
4. Milman, H. A. and D. A. Cooney (1974) Biochem. J. **142**:27.
5. Lomelino, C. L. *et al.* (2017) J. Biol. Chem. **292**:19952.
6. Ruzzo, E. K. *et al.* (2013) Neuron. **80**:429.
7. Hawkins, R. A. *et al.* (2006) J. Nutr. **136**:218S.
8. Haskell, C. M. *et al.* (1969) Biochem. Pharmacol. **18**:2578.
9. Aslanian, A. M. *et al.* (2001) Biochem. J. **357**:321.
10. Su, N. *et al.* (2008) Pediatr. Blood Cancer. **50**:274.
11. Ye, J. *et al.* (2010) EMBO J. **29**:2082.
12. Pieters, R. *et al.* (2011) Cancer **117**:238.
13. Lorenzi, P. L. *et al.* (2008) Mol. Cancer Ther. **7**:3123.
14. Dufour, E. *et al.* (2012) Pancreas. **41**:940.
15. Du, F. *et al.* (2019) Cell Death Dis. **10**:239.

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