Product Data Sheet

No. 0318

NagK-8 (N-acetyl-D-glucosamine kinase, EC 2.7.1.59)

Catalog Number: 0318

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Product Description

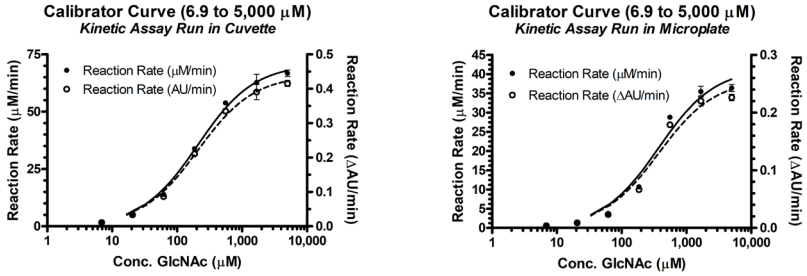
N-acetyl-D-glucosamine kinase (NagK) catalyzes the phosphorylation of N-acetyl-D-glucosamine (GlcNAc). ATP is the phosphate donor. The enzyme primarily acts on cytoplasmic GlcNAc derived from cell wall degradation as part of peptidoglycan recycling, or deglycosylated proteins. Wild-type NagK is 303 amino acids in length with a mass of 32.9 kDa.[1] NagK-8 is an engineered 33.9 kDa recombinant protein of 309 residues, which can be used to quantify GlcNAc levels in aqueous samples. NagK-8 was engineered to eliminate phosphorylation of other sugar molecules (patent pending).

Reaction:

ATP + N-acetyl-D-glucosamine → ADP + N-acetyl-D-glucosamine 6-phosphate

Instructions for Use

1. The amount of NagK-8 used in a given assay should be empirically determine by titrating the enzyme. The assay shown in the graph below was performed with NagK-8 at 275 ng/ml in a 0.2 ml reaction (55 ng).
2. Dilute the enzyme with 100 mM Tris-Cl pH 7.5. Add BSA to 100 µg/ml when diluting below 0.1 µg/ml.
3. *Assay Method 1*: Measurement of the disappearance of GlcNAc.[2] GlcNAc is mixed in buffer with and without ATP. NagK-8 is added and the reaction allowed to proceed for 30 min. The reaction is stopped by adding zinc sulfate and barium hydroxide to precipitate the phosphorylated sugars. The precipitate is removed and the amount of residual GlcNAc remaining is quantified.
4. *Assay Method 2*: Measurement of the accumulation of ADP using a coupled assay with pyruvate kinase and lactate dehydrogenase.[3] The three enzymes are mixed in reaction buffer contain ATP, phosphoenol pyruvate and NADH. The reaction is started by adding GlcNAc and the rate of decrease in absorbance at 340 nm is measured.



Figures: Calibrator curves of GlcNAc using a kinetic assay performed in a cuvette (left panel) or microplate (right panel). The assay was performed as for method 2 using a coupled enzyme assay (AthenaES, Cat. No. 0210).

Material Safety Data

FOR RESEARCH USE ONLY. NOT INTENDED OR APPROVED FOR HUMAN, DIAGNOSTICS OR VETERINARY USE. Do not ingest, swallow or inhale. Do not get in eyes, on skin, or on clothing. Wash thoroughly after handling. For complete safety information see full Material Safety Data Sheet.

Product Specifications

Characteristic	Specification
Source	Recombinant, E. coli
Content	13.5Units (500 1mL assays)
Formulation	25% glycerol storage buffer pH 7.7
Specific Activity	197 Units/mg
Purity	>95% by SDS-PAGE
Unit Definition	One Unit of NagK is defined as the amount of enzyme needed to oxidize 1 µmole of NADH (equivalent to 1 µmole phosphorylate GlcNAc) per min at 30°C.
Storage	5 years at -20°C Avoid repeated freezing and thawing. 3 months at 4°C
Km	356 µM
Vmax	94.7 µM/min
Inhibited By	ADP, EDTA, PO4 (>100 mM), glucosamine (>10 mM)
Phosphorylation of Competing Substrates	NagK-8 does not phosphorylate glucose, glucosamine, galactose, or mannose.

Ordering Information

Cat. No.	Product Name
0318	NagK-8, 13.5 Units (500 1 ml assays)
0318-5k	NagK-8, 135 Units (5,000 1 ml assays)
0318-50k	NagK-8, 1,350 Units (50,000 1 ml assays)

References

1. https://www.uniprot.org/uniprot/P75959.
2. Asensio and Ruiz-Amil. 1966. Methods Enzymol. 9:421-425.
3. Uehara and Park. 2004. J. Bact. 186:7273-7279.