Recombinant *E. coli* N-Acetyl-D-Glucosamine Kinase/NAGK

Catalog Number: 8020-GK

DESCRIPTION

Source	*E. coli*-derived Met1-Asp303 with C-terminal 6-His tag Accession # P75959
N-terminal Sequence Analysis	Met1
Predicted Molecular Mass	34 kDa

SPECIFICATIONS

SDS-PAGE	37-38 kDa, reducing conditions
Activity	Measured by its ability to phosphorylate N-acetyl-D-glucosamine. The specific activity is >10,000 pmol/min/µg, as measured under the described conditions. See Activity Assay Protocol on www.RnDSystems.com.
Endotoxin Level	<0.01 EU per 1 µg of the protein by the LAL method.
Purity	>90%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 µg per lane.
Formulation	Supplied as a 0.2 µm filtered solution in Tris, NaCl, Glycerol, Brij-35 and DTT. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 25 mM HEPES, 150 mM NaCl, 10 mM $MgCl_2$, 10 mM $CaCl_2$, pH 7.0
- Recombinant *E.coli* N-Acetyl-D-Glucosamine Kinase/NAGK (r*E.coli* NAGK) (Catalog # 8020-GK)
- Adenosine triphosphate (ATP) (Sigma, Catalog # A7699), 10 mM stock in deionized water
- N-acetyl-α-D-glucosamine (GlcNAc) (Calbiochem, Catalog # 1079), 1 M stock in deionized water
- Universal Kinase Activity Kit (Catalog # EA004)
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute 1 mM Phosphate Standard provided by the Universal Kinase Activity Kit by adding 40 µL of the 1 mM Phosphate Standard to 360 µL of Assay Buffer for a 100 µM stock.
2. Prepare standard curve by performing six one-half serial dilutions of the 100 µM Phosphate stock in Assay Buffer. The standard curve has a range of 0.078 to 5 nmol per well.
3. Prepare a reaction mixture composed of 0.5 mM ATP and 12.5 mM GlcNAc in Assay Buffer.
4. Dilute Coupling Phosphatase 4 (supplied in kit) to 10 µg/mL in Assay Buffer.
5. Dilute r*E.coli* NAGK to 1 µg/mL in Assay Buffer.
6. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of Assay Buffer.
7. Load 20 µL of the 1 µg/mL r*E.coli* NAGK into the plate. Include a control containing 20 µL of Assay Buffer.
8. Add 10 µL of 10 µg/mL Coupling Phosphatase 4 to the wells, excluding the standard curve.
9. Add 20 µL of reaction mixture to the wells, excluding the standard curve.
10. Cover the plate with a plate sealer and incubate at room temperature for 10 minutes.
11. Add 30 µL of the Malachite Green Reagent A to all wells. Mix briefly.
12. Add 100 µL of deionized water to all wells. Mix briefly.
13. Add 30 µL of the Malachite Green Reagent B to all wells. Mix and incubate for 20 minutes at room temperature.
14. Read plate at 620 nm (absorbance) in endpoint mode.
15. Calculate specific activity:

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

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

** Under these conditions, the coupling rate is 0.475.

Final Assay Conditions

Per Reaction:

- r*E.coli* NAGK: 0.020 µg
- Coupling Phosphatase 4: 0.1 ug
- ATP: 0.2 mM
- GlcNAc: 5 mM

PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. 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, -70 °C as supplied. ● 3 months, -70 °C under sterile conditions after opening.

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BACKGROUND

N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc) are repeating sugar units of peptidoglycan, the major component of bacterial cell wall structure and a drug target of various antibiotics including penicillin (1). Recently, interest has been generated regarding cell wall peptidoglycan catabolism, because as much as 50% of the peptidoglycan is turned over in one generation of bacterial growth (2). N-acetylglycosamine kinase (nagK) is a key enzyme for the recycling of GlcNAc in *E. coli* (3). Due to its high activity, it can be used for efficient conversion of GlcNAc to GlcNAc-6-phosphate. The enzyme is assayed using a phosphatase-coupled kinase assay (4).

References:

1. Plumbridge, J. (2009). J. Bacteriol. **191**:5641.
2. Reith, J. *et al* (2011). J. Bacteriol. **193**:5386.
3. Uehara, T. and Park, J.T.(2011) J. Bacteriol. **186**:7273.
4. Wu, Z.L. (2011) PLoS One **6**:e23172.

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