Recombinant Human Guanylate Kinase

Catalog Number: 9267-GU

DESCRIPTION

Source	*E. coli*-derived human Guanylate Kinase protein Ser2-Ala197, with an N-terminal Met and 6-His tag Accession # Q16774.2
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	23 kDa

SPECIFICATIONS

SDS-PAGE	24 kDa, reducing conditions
Activity	Measured by its ability to transfer phosphate from ATP to GMP. The specific activity is >7,500 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 and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Universal Kinase Activity Kit (Catalog # EA004)
- 10X Assay Buffer (supplied in kit): 250 mM HEPES, 1.5 M NaCl, 100 mM $MgCl_2$, 100 mM $CaCl_2$, pH 7.0
- Recombinant Human Guanylate Kinase (rhGUK-1) (Catalog # 9267-GU)
- Guanosine 5'-monophosphate (GMP) (Sigma, Catalog # G8377), 10 mM stock in deionized water
- 96-well Clear Plate (Catalog # DY990)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Prepare 1X Assay Buffer by diluting 10X stock 10 fold with deionized water.
2. Dilute 1 mM Phosphate Standard by adding 40 µL of the 1 mM Phosphate Standard to 360 µL of 1X Assay Buffer for a 100 µM stock. This is the first point of the standard curve.
3. Continue standard curve by performing six one-half serial dilutions of the 100 µM Phosphate stock in 1X Assay Buffer. The standard curve has a range of 0.078 to 5 nmoles per well.
4. Prepare a reaction mixture containing 0.2 mM ATP (supplied in kit) and 0.2 mM GMP in 1X Assay Buffer.
5. Dilute rhGUK-1 to 0.0667 µg/mL 1X Assay Buffer.
6. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of 1X Assay Buffer.
7. Load 15 µL of the 0.0667 µg/mL rhGUK-1 into empty wells of the same plate as the curve. Include a Control containing 15 µL of 1X Assay Buffer.
8. Add 25 µL of reaction mixture to all wells, excluding standard curve.
9. Seal plate and incubate at room temperature for 30 minutes.
10. Dilute Coupling Phosphatase 4 (supplied in kit) to 10 µg/mL 1X Assay Buffer.
11. Add 10 µL of 10 µg/mL Coupling Phosphatase 4 to wells containing enzyme and control, excluding the standard curve.
12. Seal plate and incubate at room temperature for 5 minutes.
13. Add 30 µL of the Malachite Green Reagent A to all wells. Mix briefly.
14. Add 100 µL of deionized water to all wells. Mix briefly.
15. Add 30 µL of the Malachite Green Reagent B to all wells. Mix and incubate for 20 minutes at room temperature.
16. Read plate at 620 nm (absorbance) in endpoint mode.
17. 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.
**Incubation time is 35 minutes according to the above protocol.
***Both ADP and GDP can be digested by Coupling Phosphatase 4 and release one unit of phosphate.

Final Assay Conditions

Per Reaction:

- rhGUK-1: 0.001 µg
- Coupling Phosphatase 4: 0.1 µg
- ATP: 0.1 mM
- GMP: 0.1 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.

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BACKGROUND

GUK1, also known as GMP kinase, belongs to the guanylate kinase family. This protein exists as a monomer and catalyzes the ATP-dependent conversion of GMP to GDP, thereby playing an essential role in the recycling of GMP (1). Via its catalytic activity, GUK1 participates in regulating the supply of guanine nucleotides including cGMP to signal transduction pathways (2). GUK1 is widely expressed. Overexpression of GUK1 is associated in pituitary adenomas (3). The enzymatic activity of the recombinant human GUK1 is measured using a phosphatase-coupled method (4).

References:

1. Brady, W.A. *et al.* (1996) J. Bio.Chem. **271**:16734.
2. Fitzgibbon, J. *et al.* (1996) FEBS Letters **385**:185.
3. Da Rocha, A.A. *et al.* (2006) Pituitary **9**:83.
4. Wu, Z.L. (2011) PLoS ONE **6**:e23172.

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