Recombinant Human Fructosamine-3-kinase/FN3K

Catalog Number: 9155-FU

DESCRIPTION

Source	*E. coli*-derived human Fructosamine-3-kinase/FN3K protein Glu2-Lys309, with N-terminal Met and 6-His tag Accession # Q9H479
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	36 kDa

SPECIFICATIONS

SDS-PAGE	37 kDa, reducing conditions
Activity	Measured by its ability to phosphorylate 1-deoxy-1-morpholino-D-fructose. The specific activity is >30 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	>90%, 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

- 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 Fructosamine-3-kinase/FN3K (rhFN3K) (Catalog # 9155-FU)
  Substrate: 1-Deoxy-1-morpholino-D-fructose (Sigma, Catalog # D6149), 40 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 provided by the Universal Kinase Activity kit 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. Complete the 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 nmol per well.
4. Prepare a reaction mixture containing 0.4 mM ATP (supplied in kit) and 2 mM 1-Deoxy-1-morpholino-D-fructose in 1X Assay Buffer.
5. Dilute rhFN3K to 33.3 µg/mL in 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 33.3 µg/mL rhFN3K 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 the wells, excluding the standard curve.
9. Seal plate and incubate at 37 °C for 30 minutes.
10. Dilute Coupling Phosphatase 4 (supplied in kit) to10 µg/mL in 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:

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

*Derived from the phosphate standard curve using linear or 4-parameter fitting and adjusted for Control.
**Decoupled reaction (use incubation time of 35 minutes).

Final Assay Conditions

Per Reaction:

- rhFN3K: 0.5 µg
- Coupling Phosphatase 4: 0.1 µg
- ATP: 0.2 mM
- 1-Deoxy-1-morpholino-D-fructose: 1 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, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after opening.

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BACKGROUND

Nonenzymatic glycation (Maillard reaction) is a process in which glucose and other sugars react spontaneously with amine-containing molecules, such as proteins (1). These reactions proceed through many stages, starting with glucosylamines (Schiff bases), fructosamines (Amadori compounds) (2), and aminoaldoses (Heyns compounds) (3) and ultimately lead to the formation of irreversible end products, which include crosslinks, aromatic heterocycles, and oxidized compounds (4), that are designated collectively as advanced glycation end products (AGEs) (5). The concentrations of these products are elevated in diabetes, and important in the etiology of diabetes complications (6, 7). Fructosamine-3-phosphokinase (FN3K) is a kinase that phosphorylates fructoselysine and a wide variety of fructosamines, therefore removing these AGEs (8-10). Lost the protective enzymatic activity of FN3K is also associated with the oncology of colorectal cancer (11). FN3K is particularly active in brain, heart, kidney, and skeletal muscle and in erythrocytes (12,13). The kinase activity of recombinant human PFKFB3 was assayed using a phosphatase-coupled method (14).

References:

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