Recombinant Human Pyridoxal Kinase/PDXK

Catalog Number: 8658-PK

DESCRIPTION

Source	*E. coli*-derived Glu2-Leu312, with N-terminal Met and 6-His tag Accession # O00764
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	36 kDa

SPECIFICATIONS

SDS-PAGE	36 kDa, reducing conditions
Activity	Measured by its ability to phosphorylate Pyridoxal Hydrochloride. The specific activity is >85 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	>80%, 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 and DTT. 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 Pyridoxal Kinase/PDXK (rhPDXK) (Catalog # 8658-PK)
- ATP (supplied in kit): 10 mM
- Pyridoxal Hydrochloride (Sigma, Catalog # P6155), 50 mM 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 with deionized water.
2. Dilute 1 mM Phosphate Standard supplied in 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. 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 nmol per well.
4. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of 1X Assay Buffer.
5. Prepare a reaction mixture containing 0.4 mM ATP and 2 mM Pyridoxal Hydrochloride in 1X Assay Buffer.
6. Dilute Coupling Phosphatase 4 (supplied in kit) to 10 µg/mL in 1X Assay Buffer.
7. Dilute rhPDXK to 133 µg/mL in 1X Assay Buffer.
8. Load 15 µL of the 133 µg/mL rhPDXK into empty wells of the same plate as the curve. Include a control containing 15 µL of 1X Assay Buffer.
9. Add 10 µL of 10 µg/mL Coupling Phosphatase 4 to wells containing enzyme and control, excluding the standard curve.
10. Add 25 µL of reaction mixture to the wells, excluding the standard curve.
11. Seal plate and incubate at room temperature for 10 minutes.
12. Add 30 µL of the Malachite Green Reagent A to all wells.
13. Add 100 µL of deionized water to all wells. Mix briefly.
14. Add 30 µL of the Malachite Green Reagent B to all wells. Mix and incubate for 20 minutes at room temperature.
15. Read plate at 620 nm (absorbance) in endpoint mode.
16. Calculate specific activity:

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

*Derived from the phosphate standard curve using linear or 4-parameter fitting and adjusted for Control
**The coupling rate is 0.475 under these conditions.

Final Assay Conditions

Per Well:

- rhPDXK: 2.0 µg
- Coupling Phosphatase 4: 0.1 µg
- ATP: 0.2 mM
- Pyridoxal Hydrochloride: 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, -70 °C as supplied. • 3 months, -70 °C under sterile conditions after opening.

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BACKGROUND

Pyridoxal Kinase (PDXK) phosphorylates vitamin B6, a step required for the conversion of vitamin B6 to pyridoxal-5-phosphate (PLP), an important cofactor in intermediary metabolism (1). PLP is involved in many aspects of macronutrient metabolism, neurotransmitter synthesis, histamine synthesis, as well as hemoglobin synthesis, function, and gene expression (2). PLP generally serves as a co-enzyme for many reactions and can help facilitate decarboxylation, transamination, racemization, elimination, replacement and beta-group inter-conversion reactions. In particular, PLP also is needed for the enzymatic reaction governing the release of glucose from glycogen. Overall, there are over 160 PLP-dependent enzymes, corresponding to ~4% of all known enzymatic activities (3). The classic clinical syndrome for B6 deficiency is a seborrhoeic dermatitis-like eruption, atrophic glossitis with ulceration, angular cheilitis, conjunctivitis, intertrigo, accompanied by neurologic symptoms of somnolence, confusion, and neuropathy (4).The neurotoxic effects of ophylline and ginkgotoxin are caused by their inhibitory activity against human PDXK (3, 5). Genetic variations of PDXK are associated with Parkinson disease, since PLP is involved in the biosynthesis of dopamine, a neurotransmitter linked to the disease characteristics such as motor and movement disorders (6). PDXK is also a potential drug target in the African trypanosomiasis (7). PDXK is a cytoplasmic enzyme and probably acts as a homodimer *in vivo* (8). The enzymatic activity of recombinant human CHKB is measured using a phosphatase-coupled method (9).

References:

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