Recombinant Human PANK1 His-tag

Catalog Number: 11161-PK

DESCRIPTION

Source	*E. coli*-derived human PANK1 protein Lys231-Lys598, with a N-terminal Met & 6-His tag Accession # Q8TE04.2
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	42 kDa

SPECIFICATIONS

SDS-PAGE	40 kDa, under reducing conditions.
Activity	Measured by its ability to transfer phosphate from ATP to pantothenic acid. The specific activity is >170 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, NaCl and TCEP. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Universal Kinase Activity Kit (Catalog # EA004)
- 1X Assay Buffer: 25 mM HEPES, 150 mM NaCl, 10 mM $MgCl_2$, 10 mM $CaCl_2$, pH 7.0 (10X Assay Buffer in kit EA004)
- Recombinant Human PANK1 (rhPANK1) (Catalog # 11161-PK)
- Pantothenic Acid (Sigma, Catalog # P5155), 20 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 (Phosphatase Buffer 4 that is provided in kit) 10 fold with deionized water.
2. Prepare a standard curve from the 1 mM Phosphate Standard (provided in 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. Prepare a Reaction Mixture containing 0.4 mM ATP and 2 mM Pantothenic Acid in 1X Assay Buffer.
5. Dilute Coupling Phosphatase 4 to 10 µg/mL in 1X Assay Buffer.
6. Dilute rhPANK1 to 33.34 µg/mL in 1X Assay Buffer.
7. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of 1X Assay Buffer.
8. Load 15 µL of the 33.34 µg/mL rhPANK1 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. Start the reaction by adding 25 µL of Reaction Mixture to all wells, excluding standard curve.
11. Seal and incubate plate at room temperature for 10 minutes.
12. Add 30 µL of the Malachite Green Reagent A to all wells. Mix briefly.
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.
**Under these conditions, the coupling rate is 0.475.

Final Assay Conditions

Per Reaction:

- rhPANK1: 0.5 µg
- Coupling Phosphatase 4: 0.1 µg
- ATP: 0.2 mM
- Pantothenic Acid: 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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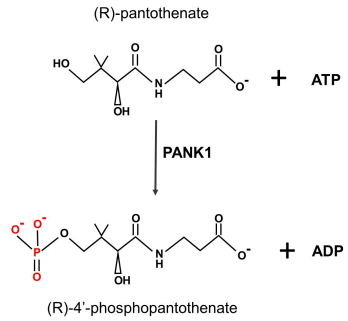
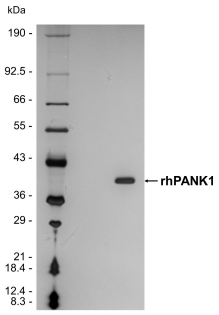
DATA

Enzyme Activity



Recombinant Human PANK1 His-tag Protein Enzyme Activity Diagram

SDS-PAGE



Recombinant Human PANK1 His-tag Protein SDS-PAGE
1 µg/lane of rhPANK1 (Catalog # 11161-PK) was resolved with SDS-PAGE under reducing (R) conditions and visualized by silver staining, showing a band at 40 kDa.

BACKGROUND

The pantothenate kinase (PANK) gene family contains four members and encodes five functional isoforms. The PANK enzymes are key to the synthesis of coenzyme A (CoA) (1,2). CoA is used by around 4% of intracellular enzymes as a co-substrate. PANK1 is the key regulatory enzyme in the CoA biosynthetic pathway and plays similar roles in both bacterial and mammalian cells (3). PANK1 expression levels are high in liver, kidney, and heart tissues (4). Mutations in PANK1 have been linked to Hallervorden-Spatz syndrome, an autosomal recessive neurodegenerative disorder caused by excessive brain iron accumulation due to the defects in CoA synthesis (5). The activity of this enzyme has been measured with a phosphatase-coupled method (6).

References:

1. Zhou, B. *et al.* (2001) Nat Genet. **28**:345.
2. Polsterm, B.J. *et al.* (2010) Gene **465**:53.
3. Robishaw, J.D. *et al.* (1985) Am. J. Physiol. **1**:248.
4. Rock, C.O. et al. (2000). J Biol Chem. **275**:1377.
5. Gordon, N. (2002) Eur J Paediatr Neurol. **6**:243.
6. Wu, Z.L. (2011) PLoS ONE **6**:e23172.

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