# Recombinant Human PANK2 His-tag

Catalog Number: 10664-PK

## DESCRIPTION

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| **Source** | *E. coli*-derived human PANK2 protein<br>Lys206-Pro570, with C-terminal 6-His<br>Accession # Q9BZ23.3 |
| **N-terminal Sequence Analysis** | Met-Lys206 |
| **Predicted Molecular Mass** | 41.6 kD |

## SPECIFICATIONS

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| **SDS-PAGE** | 39 kD |
| **Activity** | Measured by its ability to transfer phosphate from ATP to pantothenic acid.<br>The specific activity is >80 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)
- 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 PANK2 His-tag (rhPANK2) (Catalog # 10664-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 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. Perform six additional 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 Pantothenic Acid in 1X Assay Buffer.
5. Dilute Coupling Phosphatase 4 to 10 µg/mL in 1X Assay Buffer.
6. Dilute rhPANK2 to 66.7 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 66.7 µg/mL rhPANK2 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 with 25 µL of reaction mixture to all wells, excluding 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. 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:

- rhPANK2: 1 µg
- Coupling Phosphatase 4: 0.1 µg
- ATP: 0.2 mM
- Pantothenic Acid: 1 mM

## PREPARATION AND STORAGE

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| **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.**<br>• 6 months from date of receipt, -20 to -70 °C as supplied.<br>• 3 months, -20 to -70 °C under sterile conditions after opening. |

## DATA



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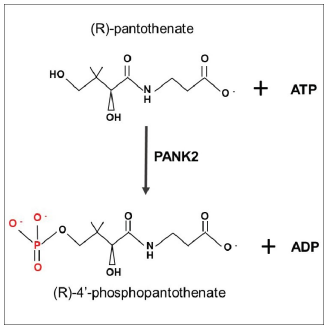
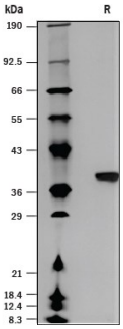
**Enzyme Activity**




**Recombinant Human PANK2 His-tag Protein Enzyme Activity Diagram** PANK2 catalyzes the first committed step in the pathway leading to the biosynthesis of coenzyme A. PANK2 is also a key regulatory enzyme in this pathway.

**SDS-PAGE**




**Recombinant Human PANK2 His-tag SDS-PAGE.** 1 µg/lane of Recombinant Human PANK2 His-tag (Catalog # 10664-PK) was resolved with SDS-PAGE under reducing (R) conditions and visualized by silver staining, showing a band at 39 kDa.

## BACKGROUND

The pantothenate kinase (PANK) gene family contains four members and encode 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. PANK2, the only member of the PANK family that resides in mitochondria, is feedback regulated by CoA and may be the master regulator of CoA synthesis (3, 4). Mutations in PANK2 results in Hallervorden-Spatz syndrome, an autosomal recessive neurodegenerative disorder caused by excessive brain iron accumulation due to the defects in CoA synthesis (1,5). 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. Rock, C.O. *et al.* (2000) J Biol Chem. **275**:1377.
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5. Orellana, D.I. *et al.* (2016) EMBO Mol Med. **8**:1197.
6. Wu, Z.L. *et al.* (2011) PLoS ONE **6**:e23172.



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