

DESCRIPTION

Source *E. coli*-derived
Leu47-Ser574, with N-terminal Met and 6-His tag
Accession # P30613

N-terminal Sequence Analysis Met

Predicted Molecular Mass 58 kDa

SPECIFICATIONS

SDS-PAGE 51-60 kDa, reducing conditions

Activity Measured by its ability to transfer phosphate from phospho(enol)pyruvic acid monosodium salt hydrate (PEP) to adenosine 5'-diphosphate sodium salt (ADP).
The specific activity is >25,000 pmol/min/ug, as measured under the described conditions.

Endotoxin Level <1.0 EU per 1 µg of the protein by the LAL method.

Purity >85%, 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**
- Assay Buffer: 0.1 M MES, 5 mM MgCl₂, 20 mM KCl, pH 6.5
 - Recombinant Human PKLR (rhPKLR) (Catalog # 8569-PK)
 - Activator: D-Fructose 1,6-bisphosphate trisodium salt hydrate (Sigma, Catalog # F6803), 50 mM stock in deionized water
 - Adenosine 5'-diphosphate sodium salt (ADP) (Sigma, Catalog # A2754), 200 mM stock in diH₂O
 - Phospho(enol)pyruvic acid monosodium salt hydrate (PEP) (Sigma, Catalog # P0564), 50 mM stock in deionized water
 - Adenosine triphosphate (ATP) (Sigma, Catalog # A7699), 10 mM stock in deionized water
 - Kinase Glo® Plus Luminescent Kinase Assay (Promega, Catalog # V3771)
 - 96-well Solid White Plate (Corning Life Sciences, Catalog # 3912)
 - Plate Reader (Model: Gemini EM by Molecular Devices) or equivalent

- Assay**
1. Follow the Kinase Glo® Plus Luminescent Kinase Assay kit directions for preparing Kinase Glo® Plus Reagent. Use the reagent immediately or freeze aliquots at -20°C for future use. (Note: This assay measures an increasing luminescent signal, which is opposite of the traditional use of the kit).
 2. Dilute rhPKLR to 0.25 µg/mL in Assay Buffer.
 3. Dilute Activator to 0.133 mM in Assay Buffer.
 4. Stimulate enzyme by combining 40 µL of 0.25 µg/mL rhPKLR and 60 µL of 0.133 mM Activator. Replace enzyme with Assay Buffer for a Control.
 5. Incubate for 5 minutes at room temperature.
 6. Prepare a substrate mixture of 5 mM ADP and 4 mM PEP in Assay Buffer.
 7. Add 100 µL of the substrate mixture to incubated rhPKLR and Control. Mix briefly.
 8. Incubate all at room temperature for 15 minutes.
 9. Dilute ATP to 100 µM in Assay Buffer. This is the first point of the standard curve. Complete the curve by performing six two-fold dilutions of 100 µM ATP in Assay Buffer. The standard curve has a range of 0.078 to 5.0 nmol per well.
 10. Load 50 µL of each dilution of the standard curve in triplicate into a solid white plate. Include a curve blank containing 50 µL of Assay Buffer.
 11. After incubation (step 8), heat reactions and control at 90-100 °C for 1 minute to stop the reaction. Cool on ice and centrifuge briefly to collect any condensation.
 12. Load 50 µL of each reaction and control in triplicate into the same plate containing the standard curve.
 13. Add 50 µL of Kinase Glo® Plus Reagent to all wells used, including standard curve.
 14. Incubate at room temperature for 10 minutes in the dark.
 15. Read plate in Luminescence endpoint mode.
 16. Calculate specific activity:

$$\text{Specific Activity (pmol/min/}\mu\text{g)} = \frac{\text{Adj. Luminescence* (RLU)} \times \text{conversion factor** (nmol/RLU)} \times 1000 \text{ pmol/nmol}}{\text{Incubation time (min)} \times \text{amount of enzyme (}\mu\text{g)}}$$

*Adjusted for Control.

**Derived from the ATP standard curve using linear fitting and adjusted for Curve Blank.

Final Assay Conditions

- Per Well:
- rhPKLR: 0.0025 µg
 - ADP: 1.25 mM
 - PEP: 1.0 mM
 - Activator: 20 µM

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.

BACKGROUND

Pyruvate kinases (PK) are glycolytic enzymes that catalyze the transfer of a phosphoryl group from phosphoenolpyruvate to ADP, generating ATP, the final step in the glycolysis pathway (1, 2). There are 4 isozymes of pyruvate kinase in mammals: L, R, M1 and M2. L type is the major isozyme in the liver, R is found in red cells, M1 is the main form in muscle, heart and brain, and M2 is found in early fetal tissues. R and L types are encoded in a same gene and caused by different splicing (3). The 33 amino acids from the N-terminus in the R type are placed with Met and Glu in the L type. PKLR is allosterically activated by fructose 1,6-bisphosphate (4). Mutation of PKLR is a frequent cause of hereditary non-spherocytic hemolytic anemia (5-8).

References:

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