Recombinant Human PKM2

Catalog Number: 7244-PK

DESCRIPTION

Source	*E. coli*-derived human PKM2 protein Ser2-Pro531, with N-terminal Met and 6-His tag Accession # P14618
N-terminal Sequence Analysis	No sequence observed. N-terminal His tag confirmed by Western analysis.
Predicted Molecular Mass	59 kDa

SPECIFICATIONS

SDS-PAGE	58-59 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 >12,500 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, Glycerol, Brij-35 and DTT. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 0.1 M MES, 5 mM $MgCl_2$, 20 mM KCl, pH 6.5
- Recombinant Human PKM2 (rhPKM2) (Catalog # 7244-PK)
- D-Fructose 1,6-bisphosphate trisodium salt hydrate (Sigma, Catalog # F6803), 50 mM stock in 50 mM Tris, pH 7.5
- Adenosine 5'-diphosphate sodium salt (ADP) (Sigma, Catalog # A2754), 200 mM stock in deionized water
- Phospho(enol)pyruvic acid monosodium salt hydrate (PEP) (Sigma, Catalog # P0564), 100 mM stock in deionized water
- Adenosine triphosphate (ATP) (Sigma, Catalog # A7699), 10 mM stock in deionized water
- Kinase-Glo® Plus Luminiscent Kinase Assay (Promega, Catalog # V3771)
- 96-well Solid White Plate (Corning Life Sciences, Catalog # 3912)
- Fluorescent Plate Reader (Model: SpectraMax 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 rhPKM2 to 4 µg/mL in Assay Buffer.
3. Dilute D-Fructose 1,6-bisphosphate to 40 µM in Assay Buffer.
4. Stimulate enzyme by combining equal volumes of diluted rhPKM2 and D-Fructose 1,6-bisphosphate. As a control combine equal volumes of Assay Buffer and D-Fructose 1,6-bisphosphate.
5. Incubate for 5 minutes at room temperature.
6. Dilute incubated rhPKM2 to 0.2 µg/mL in Assay Buffer. Perform an identical dilution to the control.
7. Prepare a substrate mixture of 5 mM ADP and 5 mM PEP in Assay Buffer.
8. Combine 80 µL of diluted rhPKM2 with 80 µL of the substrate mixture in microcentrifuge tubes. Also prepare a Blank by combining 80 µL of diluted control with 80 µL of substrate mixture. Mix briefly.
9. Incubate at room temperature for 15 minutes.
10. Dilute ATP to 100 µM in Assay Buffer. Perform six two-fold dilutions in Assay Buffer. The standard curve has a range of 0.078-5.0 nmol per well.
11. Load 50 µL of each dilution of the standard curve into a plate. Include a Curve Blank containing 50 µL of Assay Buffer.
12. Heat tubes to 95-100 °C for 3 minutes to stop the reaction. Cool on ice for 3 minutes and centrifuge tubes briefly to collect any condensation. Caution: overheating may lead to degradation of ATP produced.
13. Load plate with 50 µL of each reaction, in triplicate.
14. Add 50 uL of Kinase-Glo® Plus Reagent to all wells used. Incubate at room temperature for 10 minutes.
15. Read plate in Luminescence endpoint mode.
16. Calculate specific activity:

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

*Adjusted for Blank

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

Final Assay Conditions

Per Well:

- rhPKM2: 0.005 µg
- ADP: 1.25 mM
- PEP: 1.25 mM
- ATP standard curve: 0.078-5.0 nmol

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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 are glycolytic enzymes that catalyze the transfer of a phosphoryl group from phosphoenolpyruvate to ADP, generating ATP (1), the final step in the glycolysis pathway. There are two pyruvate kinase muscle isozymes, PKM1 and PKM2, caused by alternative splicing at the carboxy termini (2). PKM2 is specifically expressed in proliferating cells, such as embryonic stem cells, embryonic carcinoma cells, and various cancer cells (3, 4). Switching from PKM1 to PKM2 in tumor cells causes the shift in cellular metabolism to aerobic glycolysis, which is important for tumor cell proliferation and survival (5). In addition, PKM2 exists in two oligomeric forms: a highly active tetrameric form and nearly inactive dimeric form (6). The ratio between the two forms determines whether glucose are channeled to biosynthetic processes or used for glycolytic ATP production. The oligomerization of PKM2 is allosterically stimulated by D-fructose 1,6-biphosphate (FBP) and is inhibited by oxalate and 3,3',5-triiodo-L-thyronine (T3) (7, 8).

References:

1. Tani, K. *et al.* (1988) Gene **73**:509.
2. Noguchi, T. *et al.* (1986) J. Biol. Chem. **261**:13807.
3. Corcoran, E. *et al.* (1976). Biochim. Biophys. Acta **446**:96.
4. Brinck, U. *et al.* (1994). Virchows Arch. **424**:177.
5. Christofk, H.R. *et al.* (2008) Nature **452**: 230.
6. Dombrauckas, J.D. *et al.* (2005) Biochemistry **44**:9417.
7. Kato, H. *et al.* (1989) Proc. Natl. Acad. Sci. U.S.A. **86**:7861.
8. Ashizawa, K. *et al.* (1991) Biochemistry **30**:7105.

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