

**DESCRIPTION**

**Source** *E. coli*-derived  
Pro2-His520 with N-terminal Met and 6-His tag  
Accession # Q16875

**N-terminal Sequence Analysis** Met

**Predicted Molecular Mass** 60 kDa

**SPECIFICATIONS**

**SDS-PAGE** 57 kDa, reducing conditions

**Activity** Measured by its ability to phosphorylate fructose-6-phosphate.  
The specific activity is >70 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** >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**
- Universal Kinase Activity Kit (Catalog # EA004)
  - 10X Assay Buffer (supplied in kit): 250 mM HEPES, 1.5 M NaCl, 100 mM MgCl<sub>2</sub>, 100 mM CaCl<sub>2</sub>, pH 7.0
  - Recombinant Human PFKFB3 (rhPFKFB3) (Catalog # 8566-BP)
  - Fructose-6-Phosphate (Sigma, Catalog # F3627), 10 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. Complete the standard curve by performing six one-half serial dilutions of the 100 μM Phosphate stock using 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 in triplicate. Include a curve blank containing 50 μL of 1X Assay Buffer.
  5. Prepare reaction mixture containing 0.5 mM ATP and 2.5 mM Fructose-6-Phosphate in 1X Assay Buffer.
  6. Dilute rhPFKFB3 to 100 ng/μL in 1X Assay Buffer.
  7. Load 20 μL of the 100 ng/μL rhPFKFB3 into the plate in triplicate. Include a Control containing 20 μL of 1X Assay Buffer.
  8. Dilute Coupling Phosphatase 4 (supplied in kit) to 10 μg/mL in 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 20 μL of reaction mixture to the wells, excluding the standard curve.
  11. Incubate sealed 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:

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

\*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 Reaction:
- rhPFKFB3: 2 μg
  - Coupling Phosphatase 4: 0.1 μg
  - ATP: 0.2 mM
  - Fructose-6-Phosphate: 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.

**BACKGROUND**

6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3) is involved in both the synthesis and degradation of fructose-2,6-bisphosphate, a regulatory molecule that controls the activity of the enzymes phosphofructokinase 1 (PFK-1) and fructose 1,6-bisphosphatase (FBPase-1) to regulate glycolysis and gluconeogenesis (1-3). PFKFB3 has a 6-phosphofructo-2-kinase activity that catalyzes the synthesis of fructose-2,6-bisphosphate, and a fructose-2,6-bisphosphatase activity that catalyzes the degradation of fructose-2,6-bisphosphate. Recently, it is reported to be involved in vessel sprouting of endothelial cells (ECs) (4). ECs rely on glycolysis rather than on oxidative phosphorylation for ATP production and that loss of the glycolytic activator PFKFB3 in ECs impairs vessel formation. PFKFB3 also plays a crucial role in the progression of cancerous cells by enabling their glycolytic pathways even under severe hypoxic conditions (5, 6), which makes it a potential target for cancer therapy (7). The kinase activity of recombinant human PFKFB3 was assayed using a phosphatase-coupled method (8).

**References:**

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