

DESCRIPTION

Source *E. coli*-derived
Pro2 - Lys381, with a N-terminal Met and 6-His tag
Accession # P12277

N-terminal Sequence Analysis Met

Predicted Molecular Mass 43 kDa

SPECIFICATIONS

SDS-PAGE 43 kDa, reducing conditions

Activity Measured by its ability to phosphorylate creatine.
The specific activity is >3,500 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 >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 MgCl₂, 100 mM CaCl₂, pH 7.0
 - Recombinant Human Creatine Kinase BB (rhCKB) (Catalog # 9076-CK)
 - Creatine (Sigma, Catalog # C3630), 50 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 stocks 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. Prepare reaction mixture containing 0.4 mM ATP (supplied in kit) and 8 mM Creatine in 1X Assay Buffer.
 5. Dilute rhCKB to 3.33 ng/μL in 1X Assay Buffer.
 6. Dilute Coupling Phosphatase 4 (supplied in kit) to 10 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 3.33 ng/μL rhCKB 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. Add 25 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:
- rhCKB: 0.05 μg
 - Coupling Phosphatase 4: 0.1 μg
 - ATP: 0.2 mM
 - Creatine: 4 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

Creatine kinase (CK) catalyzes the conversion of creatine to phosphocreatine (PCr) by consuming adenosine triphosphate (ATP) and generating adenosine diphosphate (ADP). CK reaction is reversible and thus ATP can be generated from PCr and ADP (1). In tissues and cells that consume ATP rapidly, especially skeletal muscle, but also brain, photoreceptor cells of the retina, hair cells of the inner ear, spermatozoa and smooth muscle, PCr serves as an energy reservoir for the rapid buffering and regeneration of ATP in situ, as well as for intracellular energy transport by the PCr shuttle or circuit (2). Clinically, creatine kinase is assayed in blood tests as a marker of myocardial infarction (heart attack), rhabdomyolysis (severe muscle breakdown), muscular dystrophy, autoimmune myositides and acute renal failure. Creatine kinase B (CKB), can form homodimer (BB type), and heterodimer (MB type) with creatine kinase M (CKM). MB type is found in myocardium, and homodimer BB type is found in many tissues, especially brain. The recombinant human CKB is in the BB type and its activity is measured using a phosphatase-coupled method (4).

References:

1. Trask R.V. *et al.* (1988) J. Biol. Chem. **263**:17142.
2. Wallimann, T. *et al.* (1992) Biochem. J. **281**:21-40.
3. Wallimann, T. (1994) Curr. Biol. **4**:42.
4. Wu, Z.L. (2011) PLoS One **6**:e23172.