BIOMEDICAL RESEARCH SERVICE CENTER
UNIVERSITY at BUFFALO, STATE UNIVERSITY of NEW YORK

Department of Biochemistry, Attn: Dr. Lee, University at Buffalo, 3435 Main Street, Buffalo, NY 14214, USA
Tel/Fax: (716) 829-3106 Email: chunglee@buffalo.edu Web: www.bmrservice.com

Creatine Kinase (CK) Assay Kit (Cat #: E-128)

COMPONENTS: CK Reaction Solution- 0.5 ml, store at -80ºC
ATP Assay Solution- 10 ml (100 assays), store at -80ºC
4 mM EDTA- 10 ml, store at 4ºC
10x Cell Lysis Solution- 25 ml, store at 4ºC **(swirl bottle briefly prior to pipetting)**

PRODUCT DESCRIPTION: Luciferase-based CK assay allows sensitive detection. Kit components are stable for several years if stored and handled properly. The assay requires the use of a luminometer.

ATP Assay Solution: ATP Assay Solution should be stored at -80°C in aliquots after its first thawing. It should be shielded from light during assay.

Plasma/serum: Plasma or serum samples may need to be diluted with ice-cold 1x Cell Lysis Solution (from 10-fold dilution of 10x Cell Lysis Solution with dH_2O) to obtain assay linearity. Samples should be store at -80ºC.

Preparation of cell/tissue extract:

1. Wash $10^5 – 10^6$ cells twice with ice-cold phosphate-buffered saline (PBS), and remove PBS completely from the cell pellet. Cell pellet should be stored at -80ºC. Tissue sample should be washed with PBS thoroughly to remove blood cells, which can cause inconsistent assay result.
2. Prepare enough 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with ice-cold dH_2O. Add 50 – 100 µl ice-cold 1x Cell Lysis Solution to cell pellet. Extract cells by pipetting up and down (gently but thoroughly). Leave lysate on ice for 5 min with intermittent gentle agitation. If lysate is viscous, add more 1x Cell Lysis Solution and repeat pipetting. Centrifuge lysate in a refrigerated microfuge for 3 min at maximal speed. Recover supernatant for assay. Tissue is homogenized in 1x Cell Lysis Solution, and lysate is clarified by centrifugation. Use ~25 mg of tissue per 0.5 ml of 1x Cell Lysis Solution for tissue homogenization.
3. Perform protein assay to determine sample protein concentration. Normalize sample protein concentration by diluting with ice-cold 1x Cell Lysis Solution to 0.1 – 0.5 mg/ml. Keep protein sample on ice at all times. Freeze-thawed crude protein lysate can exhibit reduced enzyme activity. Lysate should be stored at -80ºC. Note: Do not use a buffer containing reducing agents or SDS.

CK enzyme activity assay:

1. **Setup**- Keep all thawed reagents on ice. Set up two sets of 0.5-ml microtubes for Control and Reaction for each sample to be assayed. Add 5 µl dH_2O to each tube of the Control set and 5 µl CK Reaction Solution to each tube of the Reaction set. Proceed to steps 2 − 3 to initiate CK reaction.
2. **Control set**- Add 5 µl of the first sample to 5 µl dH_2O and mix by pipetting up and down swiftly. Allow reaction to proceed for 1 min at room temperature. Terminate reaction by adding 90 µl 4mM EDTA followed by brief vortexing. Transfer tube to ice.
3. **Reaction set**- Add 5 µl of the first sample to 5 µl CK Reaction Solution and mix by pipetting up and down swiftly. Allow reaction to proceed for 1 min at room temperature. Terminate reaction by adding 90 µl 4mM EDTA followed by brief vortexing. Transfer tube to ice. Repeat steps 2 - 3 for the next sample.

RLU measurement:

1. Thaw enough ATP Assay Solution and warm to room temperature shielded from light. Blank: add 10 µl of dH_2O to a luminometer tube or well. Control and Reaction sets: add 10 µl of Control (step 2) and Reaction (step 3) of each sample to the respective luminometer tubes/wells.
2. Gently vortex thawed ATP Assay solution prior to pipetting. Use 0.1 ml ATP Assay Solution for Blank, each Control, and each Reaction tube/well. Immediately measure the relative light unit (RLU) using a luminometer.
3. Subtract Blank RLU from all Control and Reaction RLU readings. Then subtract Control RLU from Reaction RLU for each sample.
4. Sample CK activity is expressed as RLU/µg protein (for cell/tissue lysate) and RLU/µl (for plasma/serum).