BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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

COMPONENTS: CK Reaction- 0.25 ml, store at -80ºC
CK Control- 0.25 ml, store at -80ºC
ATP Assay Solution- 2x10 ml (for 100 assays), store at -80ºC **(shield solution from light during use)**
TCA Stop- 1 ml, store at 4ºC (**caution: avoid contact with skin**)
10x Sample Buffer- 25 ml, store at 4ºC

PRODUCT DESCRIPTION: Chemiluminescent detection of creatine kinase (CK)-mediated formation of ATP provides a sensitive assay of CK activity present in tissue/cell extracts, serum, and cell culture medium. The kinase enzyme activity is determined by measurement of Relative Light Unit (RLU) using a luminometer. Kit components are stable for at least one year if stored and handled properly.

Preparation of cell/tissue lysates:

1. PBS-washed cell/tissue samples should be frozen at -80°C prior to homogenization. At least ~10^5 cells or 2 mg of tissue should be used for lysate preparation. Freeze thawing, mechanical grinding, or sonication facilitates protein extraction.
2. Prepare 1x Sample Buffer by diluting 10x Sample Buffer with ice-cold dH_2O. Bring up freeze-thawed cells in 50 – 100 µl ice-cold 1x Sample Buffer by pipetting up and down. Adherent cells can be directly harvested from plate by scraping. Leave lysate on ice for 5 min with agitation. Freeze-thawed tissue is mechanically homogenized in ice-cold 1x Sample Buffer (~5 mg tissue in 0.5 ml).
3. Centrifuge lysate in a cold microfuge at ~14,000 rpm for 5 min. Supernatant is harvested and stored at -80ºC.
4. Perform protein assay to determine sample protein concentration. A suggested sample protein concentration range is 0.1 – 0.5 mg/ml. Keep lysates on ice at all times during assay. Serum samples can be used directly without dilution. *Do not use plasma for the assay.*

Enzyme assay:

1. Keep thawed CK Control, CK Reaction, and ATP Assay Solution on ice. *Do not over thaw*. Shield ATP Assay Solution from light during assay.
2. Pipette 2.5 µl CK Control to one set of 0.5-ml microtubes and 2.5 µl CK Reaction to another set of 0.5-ml microtubes. Keep all tubes on ice.
3. Initiate assay by adding 2.5 µl of the first sample to CK Control and mix solution by pipetting up and down twice. Immediately place tube in a 37°C-water bath for exactly 1 min (for cell/tissue lysate) or 5 min (for cell culture medium/serum). Remove tube from water bath and immediately add 5 µl TCA Stop. Vortex tube and place tube on ice. *Reaction time can be empirically optimized by end users.*
4. Proceed to add 2.5 µl of the first sample to CK Reaction and mix solution by pipetting up and down twice. Immediately place tube in a 37°C-water bath for exactly 1 min (for cell/tissue lysate) or 5 min (for cell culture medium/serum). Remove tube from water bath and immediately add 5 µl TCA Stop. Vortex tube and place tube on ice.
5. Follow Steps 3 - 4 for the next sample. Keep reaction time the same for all samples. All sample tubes should be kept on ice for at least 10 min after addition of TCA Stop.
6. Centrifuge control and sample tubes in a microfuge for 5 min (~14,000 rpm).
7. Remove tubes from centrifuge. Add 0.25 ml ice-cold dH_2O to each tube. Mix solution and centrifuge for 3 min.
8. Transfer 10 µl of supernatant from each tube to a clear (uncoated) 96-well plate for measurement of Relative Light Unit (RLU) using a luminometer. Add 0.1 ml ATP Assay Solution to each well and record RLU.
9. Subtract control RLU from sample RLU to obtain **ΔRLU** for each sample. CK activity is represented by **ΔRLU**, which can be normalized by protein concentration or GAPDH enzyme activity of each sample. *Increase sample protein concentration or reaction time at Steps 3-4 if low ΔRLU is obtained.*

Additional information:

- Please visit the product webpage or contact us for material SDS information.