# Creatine Kinase Microplate Assay Kit

## Basic information:

**Catalog No.:** UAK1045 **Size:** 100 Assays

*For research use only. Not for diagnostic or therapeutic procedures.*

## I. INTRODUCTION

Creatine Kinase (CK), also known as phosphocreatine kinase, is an enzyme that catalyzes the transfer of one phosphate group from ATP to creatine generating phosphocreatine, an important energy reservoir in muscle and brain tissue. CK is a dimeric protein made up of B (brain) and M (muscle) subunits. Three isoenzymes, CK-MM, CK-MB, and CK-BB, have been observed. CK levels are elevated in various pathological conditions including myocardial infarction, rhabdomyolysis, muscular dystrophy, and renal failure.

Creatine Kinase Activity Microplate Assay Kit provides a simple and direct procedure for measuring CK levels in a variety of samples such as blood, serum, and plasma. In this reaction, phosphocreatine and ADP are converted to creatine and ATP. The generated ATP is used by hexokinase to phosphorylate glucose resulting in glucose-6-phosphate, which is oxidized by NADP in the presence of glucose-6-phosphate dehydrogenase to produce NADPH and 6-phospho-D-gluconate, measured at 570 nm, proportionate to the CK activity present in the sample.

## II. KIT COMPONENTS

| Component | Volume | Storage |
|---|---|---|
| 96-Well Microplate | 1 plate | |
| Assay Buffer | 30 ml x 4 | 4 °C |
| Reaction Buffer | Powder x 1 | 4 °C |
| Substrate | Powder x 1 | -20 °C |
| Enzyme | Powder x 1 | -20 °C |
| Dye Reagent | Powder x 1 | 4 °C |

| | | |
|---|---|---|
| Standard | Powder x 1 | -20 °C |
| Positive Control | Powder x 1 | -20 °C |
| Plate Adhesive Strips | 3 Strips | |
| Technical Manual | 1 Manual | |

***Note:***

***Reaction Buffer**: add 5 ml distilled water to dissolve before use.*

***Substrate**: add 3 ml distilled water to dissolve before use.*

***Enzyme**: add 1 ml Assay Buffer to dissolve before use.*

***Dye Reagent**: add 10 ml distilled water to dissolve before use.*

***Standard**: add 1 ml distilled water to dissolve before use; then add 0.2 ml into 0.8 ml distilled water, the concentration will be 400 μmol/L.*

***Positive Control**: add 1 ml distilled water to dissolve before use, then add 0.5 ml into 0.5 ml distilled water. Store at -20 °C.*

## III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 570 nm
2. Distilled water
3. Pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

## IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5 × $10^6$ cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 10s, repeat 30 times); centrifuged at 10000g

4 °C for 15 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 10000g 4 °C for 15 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum or plasma samples

Detect directly.

## V. ASSAY PROCEDURE

Warm the Reaction Buffer, Substrate to room temperature before use.

Add following reagents in the microplate:

| Reagent | Sample | Blank | Standard | Positive Control |
| --- | --- | --- | --- | --- |
| Reaction Buffer | 50 µl | 50 µl | -- | 50 µl |
| Sample | 10 µl | -- | -- | 10 µl |
| Distilled water | -- | 10 µl | -- | -- |
| Substrate | 30 µl | 30 µl | -- | 30 µl |
| Enzyme | 10 µl | 10 µl | -- | 10 µl |
| Mix, cover the plate adhesive strip, put the plate into the convection oven, incubate at 37 °C for 5 minutes. | | | | |
| Standard | -- | -- | 100 µl | -- |
| Dye Reagent | 100 µl | 100 µl | 100 µl | 100 µl |
| Mix, measured at 570 nm immediately and record the absorbance. | | | | |

***Note**: if the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time.*

## VI. CALCULATION

**Unit Definition:** One unit of CK activity is defined as the enzyme produce 1 nmol NADPH per minute.

1. According to the protein concentration of sample

$CK (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / OD_{Standard} / (V_{Sample} \times C_{Protein}) / T$

$= 800 \times (OD_{Sample} - OD_{Blank}) / OD_{Standard} / C_{Protein}$

2. According to the weight of sample

$CK (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / OD_{Standard} / (V_{Sample} \times W / V_{Assay})/T$

$= 800 \times (OD_{Sample} - OD_{Blank}) / OD_{Standard} / W$

3. According to the quantity of cells or bacteria

$CK (U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / OD_{Standard}/(V_{Sample} \times N / V_{Assay})/T$

$= 800 \times (OD_{Sample} - OD_{Blank}) / OD_{Standard} / N$

4. According to the volume of serum or plasma

$CK (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / OD_{Standard} / V_{Sample} / T$

$= 800 \times (OD_{Sample} - OD_{Blank}) / OD_{Standard}$

$C_{Standard}$: the standard concentration, 400 μmol/L = 400 nmol/ml;

$V_{Standard}$: the volume of standard, 100 μl = 0.1 ml;

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, $N \times 10^4$;

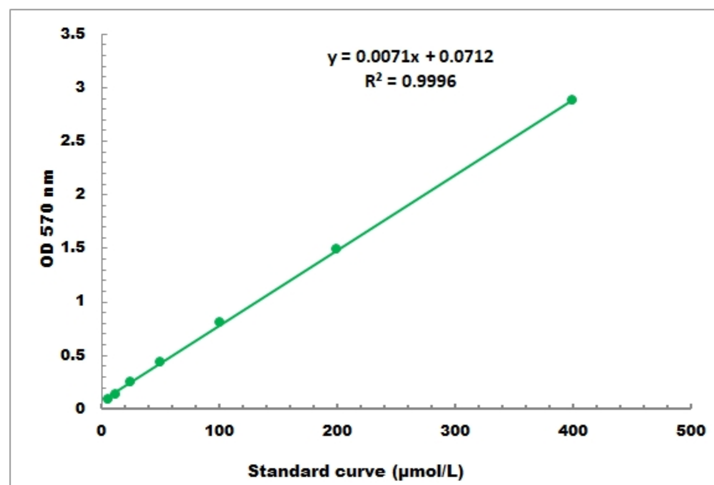
$V_{Sample}$: the volume of sample, 0.01 ml;

$V_{Assay}$: the volume of Assay buffer, 1 ml;

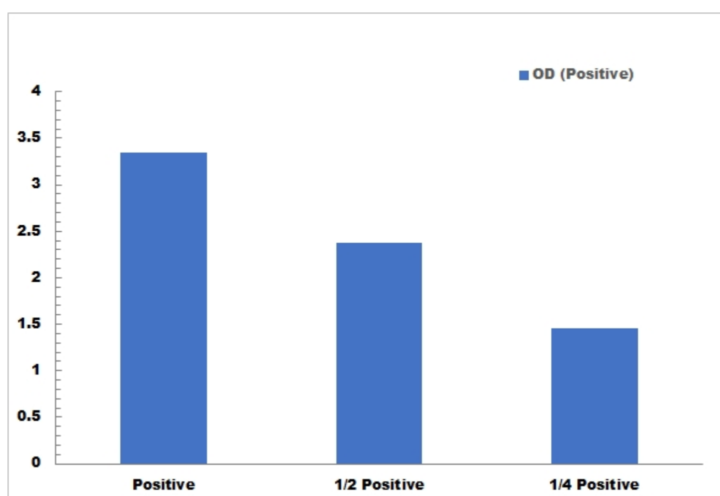
T: the reaction time, 5 minutes.

## VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.




Detection Range: 4 μmol/L - 400 μmol/L




Positive Control reaction in 96-well plate assay with decreasing Positive Control concentration



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