RayBio® Creatine Kinase Activity Assay

For the quantitative determination of Creatine Kinase activity in serum, plasma, cell lysate and other liquid sample types

Catalog #: MA-CK

User Manual
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Caution:
Extraordinarily useful information enclosed

ISO 13485:2016

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Please read the entire manual carefully before starting your experiment

I. Introduction

Creatine Kinase (CK), also known as creatine phosphokinase (CPK), is an enzyme that catalyzes the reversible phosphorylation of creatine by ATP to form phosphocreatine and ADP. Phosphocreatine is the primary storage form of high-energy phosphate in muscle tissue. There are three different CK isoenzymes: CK-BB (CPK-1), which is primarily produced by the brain and lungs; CK-MB (CPK-2), which is primarily produced by heart muscle; and CK-MM (CPK-3), which is primarily produced by skeletal muscle.

Under normal circumstances, healthy individuals have very low levels of CK in their blood. Clinically, CK levels are often measured in emergency patients with chest pain and acute renal failure. An elevation of CK levels indicates muscle damage and has been linked to conditions such as injury, rhabdomyolysis, myocardial infarction, myositis, myocarditis, malignant hyperthermia and neuroleptic malignant syndrome. Conversely, lower levels may suggest alcoholic liver disease and rheumatoid arthritis.

The RayBio® Creatine Kinase Activity Assay Kit provides a rapid (5-minute), convenient method of detecting total creatine kinase activity in biological samples. The assay is based on enzyme-coupled reactions in which CK converts phosphocreatine and ADP to creatine and ATP, the generated ATP is used to phosphorylate glucose by hexokinase (HK) to generate glucose-6-phosphate, which is then oxidized by NADP in the presence of glucose-6-phosphate dehydrogenase. The resulting NADPH is proportional to the CK activity in the sample and is measured at 340 nm.

II. General Description

The RayBio® Creatine Kinase Activity Assay is a rapid, simple, reproducible, and standardized method for measuring total CK activity in biological samples. CK catalyzes the reversible phosphorylation of ADP, in the presence of creatine phosphate, to form ATP and creatine. The enzyme hexokinase (HK) uses the ATP produced to phosphorylate glucose, generating ADP and glucose-6-phosphate (G_6P). G_6P is then oxidized to 6-phosphogluconate, resulting in the production of NADH. The rate of NADH formation, measured at 340nm, is directly proportional to CK activity.

III. Storage

Store each component following the Storage Instruction in the table below immediately upon arrival. Kit must be used within the 6-month expiration date.

IV.Reagents

Component	Size / Description	Storage Instruction
Microplate (Item A)	One 96-well (12 strips x 8 wells) plate	Store at room temperature*
Enzyme Solution	20 mL	Store at 2-8°C. Don't Freeze
Buffer Solution	4 mL	Store at 2-8°C. Don't Freeze
Sample Buffer	10 mL	Store at room temperature.
Creatine Kinase Positive Control	20 µL	Store at <-20°C

*Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

V. Additional Materials Required

1. Microplate reader capable of measuring absorbance at 340 nm at 37°C
2. Precision pipettes to deliver 2 µl to 1 ml volumes
3. Distilled or deionized water
4. Tubes to prepare sample dilutions

VI. Reagent Preparation

Keep kit reagents at room temperature during reagent preparation steps.

A. Reaction Solution

1. For one 96-well plate, mix 20ml of Enzyme Solution and 4ml of Buffer Solution to prepare the Reaction Solution. Mix well.

B. Positive Control

Creatine Kinase (CK) positive control: add 1µL of Creatine Kinase Positive Control into 999 µL Sample Buffer. Mix well.

C. Sample Preparation

1. Cell lysates can be prepared as follows:

For attached cells, remove supernatant from cell culture, wash cells twice with cold 1X PBS (for suspension cells, pellet the cells by spinning down the cells at 1,000 x g for 10 min) making sure to remove any remaining PBS before adding lysis buffer. Solubilize the cells at $2x10^7$ cells/ml in lysis buffer containing protease inhibitors. Pipette up and down to resuspend cells and rock the lysates gently at 2–8 °C for 30 minutes. Transfer extracts to microfuge tubes and centrifuge at 14,000 x g for 10 minutes. It is recommended that sample protein concentrations should be determined using a total protein assay.

Lysates should be used immediately or aliquot and stored at -70°C. Thawed lysates should be kept on ice prior to use.

General tips for preparing lysate samples can be viewed on the online Resources page of the website: https://www.raybiotech.com/tips-on-sample-preparation/

2. Plasma or serum samples:

Centrifuge to remove particulate matter.
No dilution/Neat is recommended for a typical Serum/Plasma sample.

Note: Optimal sample dilutions and amounts will need to be determined by each researcher empirically. Sample Buffer should be used to dilute samples. Normalize by loading equal amounts of protein per sample.

Note: If you have any questions regarding the recommended dilutions, you may contact technical support at 888-494-8555 or techsupport@raybiotech.com.

VII. Assay Procedure

1. Prepare Reaction Solution according to Section VI Reagent Preparation, Step A
2. Prepare Positive Control according to Section VI Reagent Preparation, Step B
3. Pipette 200ul of Reaction Solution into the appropriate wells of the 96-well plate and incubate at 37°C for five minutes. (samples, blanks, and positive controls are recommended to be run in duplicate)
4. Transfer 10ul of Sample, Blank (deionized water), and Positive Control to each well filled with Reaction Solution, mix and incubate at 37°C for two minutes.
5. After two minutes, read and record the absorbance (A1) at 340nm 37°C. Repeat readings every minute for the next two minutes at 37°C (A2, A3).

VIII. Calculation of Results

Calculate the mean absorbance for each set of duplicate samples and positive controls. Subtract the average blank optical density for each minute.

$A1' = A1 - A1_{Blank}$, $A2' = A2 - A2_{Blank}$, $A3' = A3 - A3_{Blank}$.

Calculate the average absorbance difference per minute $\Delta OD_{340nm}/min$.

$\Delta OD_{340nm}/min = ((A2' - A1') + (A3' - A2'))/2$.

One international Unit (U/L) is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute under defined

conditions.

CK activity (U/L)= $\frac{\Delta OD_{340nm}/min \times 210\mu l \times 1000}{3.66mM^{-1} \times 10\mu l} \times sample\ dilution$

Where: ΔOD_{340nm}/min = Average absorbance change per minute
1000 = Conversion of U/ml to U/L
3.66mM^{-1} is the adjusted extinction coefficient for NADH at 340nm with the current path length of the solution in a 96-well plate.

*CK activity SI Units (nkat/L) = CK activity (U/L)*16.67*

A. Typical Data

	A3'-A2'	A2'-A1'	ΔOD340nm/min	CK activity(U/L)
Serum	0.0045	0.0035	0.004	22.95
Plasma (EDTA)	0.003	0.0025	0.00275	15.78
Plasma (Citrate)	0.0075	0.0055	0.0065	37.29
Plasma (Heparin)	0.011	0.009	0.01	57.38
Blank (DI water)	0	0	0	0
Positive Control	0.1085	0.1085	0.1085	622.54

B. Linear Range

3.5-1500 U/L CK activity

C. Reproducibility

Intra-assay Precision (Precision within an assay):

To assess intra-assay precision, 20 wells per sample (total of 6 samples) were tested on a single plate. The intra-assay coefficient of variation was found to be 3.4%.

Inter-assay Precision (Precision between assays):

To assess inter-assay precision, 6 samples were tested in separate assays (n=6). The inter-assay coefficient of variation was found to be 5.6%.

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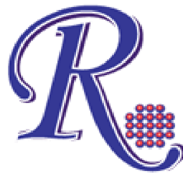
This product is for research use only.



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