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Product Information

Creatine Kinase Activity Assay Kit

Catalogue Number **MAK116**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

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.

The Creatine Kinase Activity Assay kit provides a simple and direct procedure for measuring CK levels in a variety of samples such as, serum, and plasma. In this assay, Creatine Kinase activity is determined by a coupled enzyme reaction resulting in the production of NADPH, measured at 340 nm, proportionate to the CK activity present in the sample. 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. One unit of CK is the amount of enzyme that will transfer 1.0 μmole of phosphate from phosphocreatine to ADP per minute at pH 6.0. This kit has a linear range of 30–1,800 units/L CK activity.

Components

The kit is sufficient for 100 assays in 96 well plates.

Assay Buffer Catalogue Number MAK116A	12 mL
Enzyme Mix Catalogue Number MAK116B	120 μL
Substrate Solution Catalogue Number MAK116C	1 mL
Calibrator Catalogue Number MAK116D	150 μL

Reagents and Equipment Required but Not Provided.

- Ultraviolet Spectrophotometric multiwell plate reader
- Clear 96 well flat-bottom plate suitable for use in UV absorbance assays (Catalog No. CLS3635 or equivalent).

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

This kit is shipped on dry ice. Storage at –20 °C, protected from light, is recommended.

Procedure

Sample Preparation

Blood samples should be not be hemolyzed and assayed within 4 hours of collection if stored at room temperature and 12 hours if samples are stored at 2–8 °C. Alternatively, samples can be stored at –80 °C. Frozen samples can be thawed one time and may demonstrate some loss of activity. If turbidity is observed in the samples, centrifuge samples and used clear lysate for reactions.

Tissue samples should be rinsed in phosphate-buffered saline, pH 7.4, to remove blood. Homogenize tissue (50 mg) in 200 μL of 50 mM postassium phosphate, pH 7.5, buffer. Centrifuge at 10,000 × g for 15 minutes at 2–8 °C. Use cleared supernatant for assay.

Collect cells by centrifugation at 2,000 × g for 5 minutes at 2–8 °C. For adherent cells, do not harvest using proteolytic enzymes, instead use a cell scraper. Homogenize or sonicate cells in an appropriate volume of cold 50 mM potassium phosphate, pH 7.5, buffer. Centrifuge at 10,000 × g for 15 minutes at 2–8 °C. Remove supernatant for assay.

All samples can be stored at –80 °C for up to one month.

Assay Reaction

1. This reaction can be carried out at either room temperature or 37 °C. Bring all components to room temperature or 37 °C before use. Prepare enough of the Reconstituted Reagent for each sample to be tested according to the scheme in Table 1. Each sample requires 100 µL of Reconstituted Reagent.

Table 1.
Reconstituted Reagent

Reagent	Volume
Assay Buffer	100 µL
Substrate Solution	10 µL
Enzyme Mix	1 µL

2. Transfer 110 µL of water into one well (Blank) and 100 µL of water plus 10 µL of the Calibrator into a separate well of a 96 well plate.

3. Transfer 10 µL of samples into separate wells. Add 100 µL of the Reconstituted Reagent to each sample well and tap plate to mix.

4. Incubate the samples at either room temperature or 37 °C. After 20 minutes, take the initial absorbance measurement at 340 nm $(A_{340})_{initial}$.
   Note: CK is fully activated within 20 minutes by the glutathione present in the Substrate Solution.

5. Continue to incubate the plate at either room temperature or 37 °C for 20 additional minutes. Measure the $(A_{340})_{final}$.
   Note: If the CK activity is expected to be higher than 300 units/L, measure the A_{340} at 5 minutes past the initial measurement.

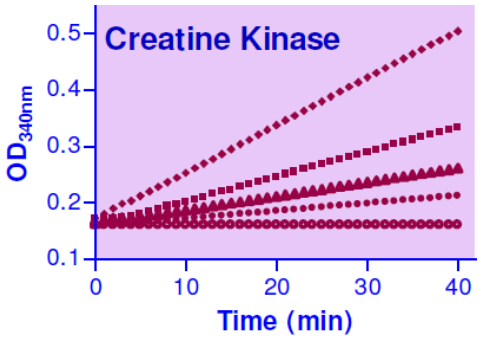
Calculations

$$\text{CK Activity (units/L)} = \frac{(A_{340})_{final} - (A_{340})_{initial}}{(A_{340})_{calibrator} - (A_{340})_{blank}} \times 150$$

where:
150 = equivalent activity (units/L) of the Calibrator when assay is read at 20 minutes and 40 minutes (20 additional minutes past initial reading).
Note: If the CK activity is expected to be higher than 300 units/L, read A_{340} at 20 minutes and again at 25 minutes. To calculate the CK activity, replace $[(A_{340})_{40\ min} - (A_{340})_{20\ min}]$ with $[(A_{340})_{25\ min} - (A_{340})_{20\ min}]$ and replace the factor 150 with 600 in the above equation.

Linear range: 30–1,800 units/L of CK activity

One unit of CK is the amount of enzyme that will transfer 1.0 µmole of phosphate from phosphocreatine to ADP per minute at pH 6.0.



Kinetics of CK reaction at 25 (solid circles), 50 (triangles), 100 (squares), and 200 (diamonds) units/L with Control (open circles).

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For UV assays, use clear plates that are UV transparent or quartz plates.
Samples with erratic readings	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
	Samples measured at incorrect wavelength	Check the equipment and filter settings

PM,LS,MAM 05/14-1