Creatine Assay Kit

Catalog Number KA1666

100 assays

Version: 03

Intended for research use only

www.abnova.com

Table of Contents

Introduction

Intended Use

Applications:

- ✓ Direct Assays: creatine in biological samples (e.g. serum, plasma, urine, saliva etc).

Features:

- ✓ High sensitivity and wide linear range. Use 10 µL sample. Linear detection range 4 to 1000 µM (colorimetric) or 0.5 to 50 µM (fluorimetric).
- ✓ Homogeneous and simple procedure. Simple “mix-and-measure” procedure allows reliable quantitation of creatine within 30 minutes.

Principle of the Assay

CREATINE is present in vertebrates and helps to supply energy to muscle. In humans and animals, approximately half of creatine originates from food (mainly from fresh meat). Creatine supplementation has been investigated as a possible therapeutic approach for the treatment of muscular, neuromuscular, neurological and neurodegenerative diseases.

Simple, direct and automation-ready procedures for measuring creatine are popular in research and drug discovery. Creatine Assay Kit is based on enzymatic reactions leading to formation of a pink colored product. The optical density at 570 nm or fluorescence intensity at λem/ex = 590/530 nm is directly proportional to the creatine concentration in the sample.

General Information

Materials Supplied

List of component

Component	Amount
Assay Buffer	20 mL
Enzyme A	120 µL
Enzyme B	220 µL
Standard: 20 mM creatine	400 µL
Dye Reagent	220 µL

Storage Instruction

Store all components at -20°C. Shelf life: 12 months after receipt.

Materials Required but Not Supplied

Pipeting devices, and clear flat-bottom 96-well plates and optical density plate reader for colorimetric assays; black flat-bottom 96-well plate and fluorescence intensity plate reader for fluorimetric assays.

Precautions for Use

- Precautions

✓ Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents.

Assay Protocol

Sample Preparation

SH-group containing reagents (e.g. mercaptoethanol, DTT) and EDTA may interfere with this assay and should be avoided in sample preparation. Solid samples can be extracted by homogenization in distilled water (dH_2O) and filtered or centrifuged. Liquid samples (e.g. serum, plasma and urine) can be assayed directly.

Assay Procedure

✓ Colorimetric Procedure

1. Standards and Samples. Equilibrate all components to room temperature. Briefly centrifuge tubes before opening. Prepare a 1000 µM creatine Standard Premix by mixing 15 µL of the 20 mM Standard and 285 µL dH_2O. Dilute Standard as follows.

No	Premix + dH_2O	Vol (µL)	Creatine (µM)
1	100µL + 0µL	100	1000
2	60µL + 40µL	100	600
3	30µL + 70µL	100	300
4	0µL + 100µL	100	0

   Transfer 10 µL standards into separate wells of a clear, flat-bottom 96- well plate.
   Transfer 10 µL of each sample into two separate wells, one serving as a sample blank well (R_{BLANK}) and one as a sample well (R_{SAMPLE}).
2. Enzyme Reaction. For each standard and sample well, prepare Working Reagent by mixing 90 µL Assay Buffer, 1 µL Enzyme A, 1 µL Enzyme B and 1 µL Dye Reagent. Add 90 µL Working Reagent to the four Standards and the Sample Wells.
   Prepare blank control reagent by mixing 90 µL Assay Buffer, 1 µL Enzyme B and 1 µL Dye Reagent (i.e. no Enzyme A). Add 90 µL Blank control reagent only to the Sample Blank Wells. Tap plate to mix. Incubate 30 min at room temperature.
3. Read OD_{570nm}.

✓ Fluorimetric Procedure

The fluorimetric procedure is the same as for the colorimetric assay, except that (1) the detection range is up to 50 µM creatine and (2) a black, flat bottom 96-well plate is used. Creatine standards of 0, 15, 30 and 50 µM are prepared. After incubation for 30 min at room temperature, read fluorescence intensity at λex = 530 nm and λem = 590 nm.

Data Analysis

Calculation of Results

Subtract the standard values from the blank value (#4) and plot the ΔOD or ΔF against standard concentrations. Determine the slope and calculate the creatine concentration of Sample,

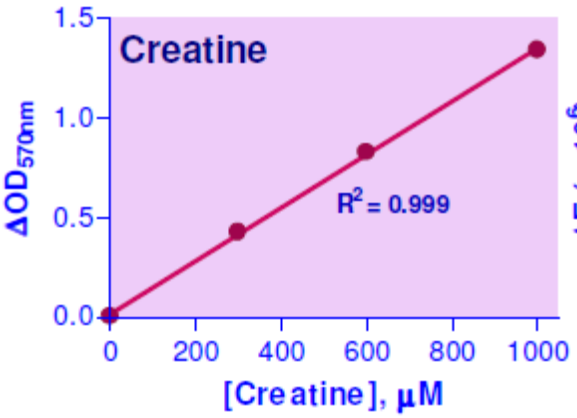
$$[\text{Creatine}] = \frac{R_{SAMPLE} - R_{BLANK}}{\text{Slope}(\mu M^{-1})} \times n\,(\mu M)$$

R_{SAMPLE} and R_{BLANK} are optical density or fluorescence intensity readings of the Sample and Sample Blank, respectively. n is the sample dilution factor.

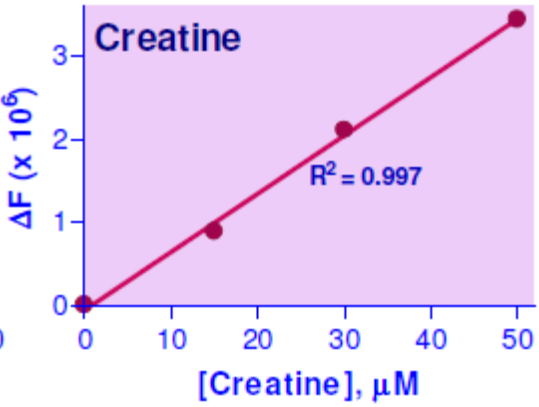
Note: if the calculated creatine concentration is higher than 1000 µM in the colorimetric assay or 50 µM in the fluorimetric assay, dilute sample in dH_2O and repeat assay. Multiply result by the dilution factor n.

Conversions: 1000 µM creatine equals 13.1 mg/dL or 131 ppm.

✓ Standard Curve in 96-well plate assay



96-well colorimetric assay



96-well fluorimetric assay

Resources

References

1. Jabs, C.M. et al (1988). Plasma creatine determination using a luminescence method. Biochem Med Metab Biol. 39(3):267-272.
2. Anderson, D.R. et al (1957). Determination of creatine in biological fluids. Biochem J. 67(2): 258-262.
3. Delanghe, J. et al (1986). Early diagnosis of acute myocardial infarction by enzymatic urinary creatine determination. Clin Chem. 32(8):1611.