EnzyChrom™ Creatine Assay Kit (ECRT-100)

Quantitative Colorimetric/Fluorimetric Determination of Creatine

DESCRIPTION

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. BioAssay Systems' creatine assay is based on enzymatic reactions leading to formation of a pink colored product. The optical density at 570 nm or fluorescence intensity at $\lambda_{em/ex}$ = 590/530 nm is directly proportional to the creatine concentration in the sample.

KEY 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.

APPLICATIONS

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

KIT CONTENTS (100 tests in 96-well plates)

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

Storage conditions. The kit is shipped chilled. Store all components at -20℃. Shelf life: 12 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

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.

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 λ_{ex} = 530 nm and λ_{em} = 590 nm.

CALCULATION

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,

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

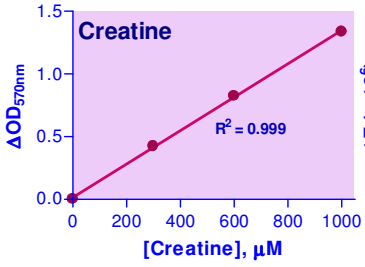
R_{SAMPLE} and R_{BLANK} are optical density or fluorescence intensity readings of the Sample and Sample Blank, respectively. *n* is the sample dilution factor.

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*.

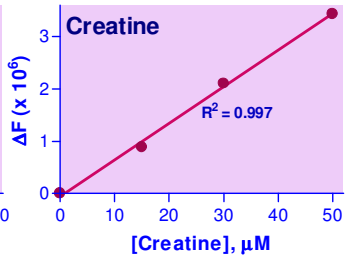
Conversions: 1000 µM creatine equals 13.1 mg/dL or 131 ppm.

MATERIALS REQUIRED, BUT NOT PROVIDED

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.



96-well colorimetric assay



96-well fluorimetric assay

LITERATURE

1. Jabs, C.M. et al (1988). Plasma creatine determination using a luminescence method. Biochem Med Metab Biol. 39(3):267-272.

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3. Delanghe, J. et al (1986). Early diagnosis of acute myocardial infarction by enzymatic urinary creatine determination. Clin Chem. 32(8):1611.