Creatine Assay Kit

Catalog Number KA1666
100 assays
Version: 03

Intended for research use only
Table of Contents

Introduction .......................................................................................................................... 3
  Intended Use .................................................................................................................. 3
  Principle of the Assay .................................................................................................... 3

General Information .......................................................................................................... 4
  Materials Supplied ......................................................................................................... 4
  Storage Instruction ......................................................................................................... 4
  Materials Required but Not Supplied ............................................................................. 4
  Precautions for Use ........................................................................................................ 4

Assay Protocol .................................................................................................................. 5
  Sample Preparation ......................................................................................................... 5
  Assay Procedure ............................................................................................................ 5

Data Analysis ..................................................................................................................... 6
  Calculation of Results ..................................................................................................... 6

Resources .......................................................................................................................... 7
  References ...................................................................................................................... 7
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

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Buffer</td>
<td>20 mL</td>
</tr>
<tr>
<td>Enzyme A</td>
<td>120 μL</td>
</tr>
<tr>
<td>Enzyme B</td>
<td>220 μL</td>
</tr>
<tr>
<td>Standard: 20 mM creatine</td>
<td>400 μL</td>
</tr>
<tr>
<td>Dye Reagent</td>
<td>220 μL</td>
</tr>
</tbody>
</table>

**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 (dH₂O) 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 dH₂O. Dilute Standard as follows.

<table>
<thead>
<tr>
<th>No</th>
<th>Premix + dH₂O</th>
<th>Vol (µL)</th>
<th>Creatine (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100µL + 0µL</td>
<td>100</td>
<td>1000</td>
</tr>
<tr>
<td>2</td>
<td>60µL + 40µL</td>
<td>100</td>
<td>600</td>
</tr>
<tr>
<td>3</td>
<td>30µL + 70µL</td>
<td>100</td>
<td>300</td>
</tr>
<tr>
<td>4</td>
<td>0µL + 100µL</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

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<sub>BLANK</sub>) and one as a sample well (R<sub>SAMPLE</sub>).

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<sub>570nm</sub>.**

✔ **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<sub>ex</sub> = 530 nm and λ<sub>em</sub> = 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,

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

\( R_{\text{SAMPLE}} \) and \( R_{\text{BLANK}} \) 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 \( \mu M \) in the colorimetric assay or 50 \( \mu M \) in the fluorimetric assay, dilute sample in \( dH_2O \) and repeat assay. Multiply result by the dilution factor \( n \).

Conversions: 1000 \( \mu M \) creatine equals 13.1 mg/dL or 131 ppm.

✓ Standard Curve in 96-well plate assay
Resources

References