Creatine Assay Kit (Colorimetric)

Creatine Assay Kit (Colorimetric) is a detection kit for the quantification of Creatine in Serum, plasma, urine and saliva.

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Package: 100 tests

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INTRODUCTION

Creatine is an organic compound with the nominal formula $(H_2N)(HN)CN(CH_3)CH_2CO_2H$. This species exists in various modifications (tautomers) in solution. Creatine is found in vertebrates where it facilitates recycling of adenosine triphosphate (ATP), the energy currency of the cell, primarily in muscle and brain tissue. Recycling is achieved by converting adenosine diphosphate (ADP) back to ATP via donation of phosphate groups. Creatine also acts as a buffer. [Provide by Wikipedia: Creatine]

PRINCIPLE OF THE ASSAY

This Creatine Assay Kit (Colorimetric) is a simple assay that measures the amount of creatine present in biological samples. This assay is based on enzymatic reactions leading to formation of a pink colored product. The optical density at 570 nm or fluorescence intensity at lem/ex = 590/530 nm is directly proportional to the creatine concentration in the sample.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped on ice. Store all components at -20°C upon receiving. Shelf life: 12 months after receipt.

Component	Quantity	Storage information
Assay Buffer	20 mL	-20°C
Enzyme A	120 µL	-20°C
Enzyme B	220 µL	-20°C
Dye Reagent	220 µL	-20°C
Standard (20 mM creatine)	400 µL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 570 nm
- Fluorescence microplate reader capable of reading excitation at 530 nm and emission at 590 nm.
- Clear or black flat-bottom 96 well microplate
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- 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.
- SH-group containing reagents (E.g., mercaptoethanol and DTT) and EDTA may interfere with this assay and should be avoided in sample preparation.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes at 4°C. Collect the serum and assay directly.

Plasma: Collect blood with heparin or citrate and centrifuge at 2000 x g for 10 minutes at 4°C. Collect the plasma layer and assay directly.

Urine: Assay directly.

Solid samples: Extracted by homogenization in distilled water and filtered or centrifuged.

Note: SH-group containing reagents (E.g., mercaptoethanol and DTT) and EDTA may interfere with this assay and should be avoided in sample preparation.

REAGENT PREPARATION

- **Working Reagent:** for each well, mixing 40 µL of Assay Buffer, 1 µL of Enzyme A, 5 µL of Substrate and 1 µL of ATP.
- **Standard:** Prepare a 1000 µM creatine Standard Premix by mixing 15 µL of the 20 mM Standard and 285 µL of distilled water. Dilute Standard as follows.

Standard tube	Creatine (µM)	Distilled water (µL)	1000 µM Standard Premix (µL)
S1	1000	0	100
S2	600	40	60
S3	300	70	30
S4	0	100	0

ASSAY PROCEDURE

Equilibrate reagents to room temperature. Briefly centrifuge tubes before use.

Colorimetric Assay

1. Add **10 µL** of diluted **Standards** into wells of clear bottom 96-well microplate.
2. Add **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})**
3. Add **90 µL** of **Working Reagent** to four Standard and sample wells. Tap lightly to mix.
4. Add **90 µL** of **Blank Control Reagent** to the sample blank wells. Tap lightly to mix.
5. Incubate for **30 minutes** at **room temperature**.

6. Read the absorbance at O.D. 570 nm.

Fluorimetric Assay

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 minutes at room temperature, read fluorescence intensity at λex = 530 nm and λem = 590 nm.

CALCULATION OF RESULTS

1. Subtract the standard values from the blank value (S4) and plot the ΔOD or ΔF against standard concentrations. Determine the slope and calculate the creatine concentration of Sample,

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

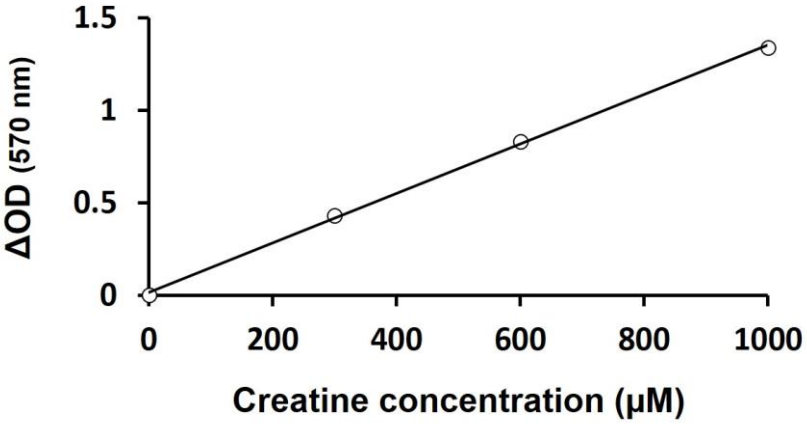
   Note:

   - R_{SAMPLE}, R_{BLANK}: the O.D. 570 nm values or fluorescence intensity of the sample and blank.
   - If the calculated creatine concentration of a sample is higher than 1000 µM in the Colorimetric Assay or 50 µM in the Fluorimetric Assay, dilute sample in Assay Buffer and repeat the assay. Multiply result by the dilution factor, n.

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

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Creatine Assay Kit (Colorimetric). One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

4 μM