Explore. Bioreagents.

# Creatine Microplate Assay Kit <br> Cat \#: orb707351 (manual) 

Detection and Quantification of Creatine Content in Urine, Serum, Plasma, Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

## INTRODUCTION

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.

Creatine Microplate Assay Kit provides an accurate, convenient measure of creatine concentration in biological fluids such as serum, urine or CSF. In the assay, creatine is converted to sarcosine, which is specifically oxidized to produce a product which reacts with a probe to generate red color, can be measured at a colorimetric readout at 546 nm .

## KIT COMPONENTS

| Component | Volume | Storage |
| :---: | :---: | :---: |
| $96-$ Well Microplate | 1 plate |  |
| Reaction Buffer | 20 ml x 1 | $4{ }^{\circ} \mathrm{C}$ |
| Enzyme | Powder x 1 | $-20^{\circ} \mathrm{C}$, keep in dark |
| Dye Reagent | Powder x 1 | $-20^{\circ} \mathrm{C}$, keep in dark |
| Standard | Powder x 1 | $4{ }^{\circ} \mathrm{C}$ |
| Plate Adhesive Strips | 3 Strips |  |
| Technical Manual | 1 Manual |  |

## Note:

Enzyme: add 9 ml Reaction Buffer to dissolve before use.
Dye Reagent: add 10 ml Reaction Buffer to dissolve before use.
Standard: add 1 ml distilled water to dissolve before use; then add $50 \mu \mathrm{l}$ into $950 \mu \mathrm{l}$ distilled water. The concentration will be $5 \mathrm{mmol} / \mathrm{L}$.

## MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 546 nm
2. Distilled water
3. Pipettor, multi-channel pipettor

Explore. Bioreagents.
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer

## SAMPLE PREPARATION

1. For urine, serum or plasma samples

Detect directly.

## ASSAY PROCEDURE

Warm all reagents to room temperature before use.
Add following reagents into the microplate:

| Reagent | Standard | Blank | Sample |
| :---: | :---: | :---: | :---: |
| Standard | $10 \mu \mathrm{l}$ | -- | -- |
| Distilled water | -- | $10 \mu \mathrm{l}$ | -- |
| Sample | -- | - | $10 \mu \mathrm{l}$ |
| Enzyme | $90 \mu \mathrm{l}$ | $90 \mu \mathrm{l}$ | $90 \mu \mathrm{l}$ |
| Dye Reagent | $100 \mu \mathrm{l}$ | $100 \mu \mathrm{l}$ | $100 \mu \mathrm{l}$ |
| Mix, put it in the oven, $37^{\circ} \mathrm{C}$ for 15 minutes, measured at 546 nm and record the |  |  |  |
| absorbance. |  |  |  |

## Note:

1) Perform 2 -fold serial dilutions of the top standards to make the standard curve.
2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment \& testing several doses to ensure the readings are within the standard curve range.

Explore. Bioreagents.

## CALCULATION

1. According to the volume of sample

Creatine $(\mathrm{mmol} / \mathrm{L})=\left(\mathrm{OD}_{\text {Sample }}-\mathrm{OD}_{\text {Blank }}\right) /\left(\mathrm{OD}_{\text {Standard }}-\mathrm{OD}_{\text {Blank }}\right) \times \mathrm{C}_{\text {Standard }}$

$$
=5 \times\left(\mathrm{OD}_{\text {Sample }}-\mathrm{OD}_{\text {Blank }}\right) /\left(\mathrm{OD}_{\text {Standard }}-\mathrm{OD}_{\text {Blank }}\right)
$$

CStandard: the standard concentration, $5 \mathrm{mmol} / \mathrm{L}$.

## TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.


Detection Range: $0.05 \mathrm{mmol} / \mathrm{L}-5 \mathrm{mmol} / \mathrm{L}$

