Technical Bulletin

# Creatine Assay Kit

**Catalogue number MAK494**

## Product 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. The Creatine Assay Kit is based on enzymatic reactions leading to the formation of a pink colored product. The absorbance at 570 nm or fluorescence intensity at $\lambda_{Ex}$ =530 nm/$\lambda_{Em}$ =590 nm is directly proportional to the creatine concentration in the sample.

The linear detection range of the kit is 4 to 1000 µM for the colorimetric assay and 0.5 to 50 µM for the fluorometric assay. The kit is suitable for creatine concentration determination in biological samples such as serum, plasma, urine, and saliva.

## Components

The kit is sufficient for 100 colorimetric or fluorometric assays in 96-well plates.

- Assay Buffer 20 mL
  Catalogue Number MAK494A
- Enzyme A 120 µL
  Catalogue Number MAK494B
- Enzyme B 220 µL
  Catalogue Number MAK494C
- Dye Reagent 220 µL
  Catalogue Number MAK494D
- Standard (20 mM creatine) 400 µL
  Catalogue Number MAK494E

## Equipment Required but Not Provided

- Pipetting devices and accessories (for example, multichannel pipettor)
- Multiwell plate reader
- Clear flat-bottom 96-well plates for colorimetric assay or black flat-bottom 96-well plates for fluorometric assay. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes
- Dounce tissue grinder set
  (Catalogue Number D9063 or equivalent)

## Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

## Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

## Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature prior to use.

## Procedure

All Samples and Standards should be run in duplicate.

### Sample Preparation

**Note:** SH-group containing reagents (for example: mercaptoethanol, DTT, and EDTA) may interfere with this assay and should be avoided in Sample preparation

1. Solid Samples can be extracted by homogenization in purified water and filtered or centrifuged.
2. Liquid Samples (for example: serum, plasma, and urine) can be assayed directly.
3. 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 ($R_{SAMPLE}$).

### Colorimetric Standard Curve Preparation:

1. Prepare a 1000 µM Standard by mixing 15 µL of the 20 mM Standard with 285 µL of purified water.
2. Prepare Standards in 1.5 mL microcentrifuge tubes according to Table 1.

**Table 1.**
Preparation of Creatine Colorimetric Standards

| Well | 1000 µM Standard | Purified Water | Creatine (µM) |
|---|---|---|---|
| 1 | 100 µL | 0 µL | 1000 |
| 2 | 60 µL | 40 µL | 600 |
| 3 | 30 µL | 70 µL | 300 |
| 4 | 0 µL | 100 µL | 0 |

3. Mix well and transfer 10 µL of each Standard into separate wells of a clear 96-well plate.

### Fluorometric Standard Curve Preparation

1. Prepare a 500 µM Standard by mixing 10 µL of the 20 mM Standard with 390 µL of purified water.
2. Prepare Standards in 1.5 mL microcentrifuge tubes according to Table 2.

**Table 2.**
Preparation of Creatine Fluorometric Standards

| Well | 500 µM Standard | Purified Water | Creatine (µM) |
|---|---|---|---|
| 1 | 10 µL | 90 µL | 50 |
| 2 | 6 µL | 94 µL | 30 |
| 3 | 3 µL | 97 µL | 15 |
| 4 | 0 µL | 100 µL | 0 |

3. Mix well and transfer 10 µL of each Standard into separate wells of a black 96-well plate.

### Working Reagent Preparation

Mix enough reagent for the number of assays to be performed. For each Sample and Standard well, prepare 93 µL of Working Reagent according to Table 3. For each Sample Blank well, prepare 92 µL of Blank Control Reagent according to Table 3.

**Table 3.**
Preparation of Working Reagents

| Reagent | Working Reagent | Blank Control Reagent |
|---|---|---|
| Assay Buffer | 90 µL | 90 µL |
| Enzyme A | 1 µL | - |
| Enzyme B | 1 µL | 1 µL |
| Dye reagent | 1 µL | 1 µL |

Transfer 90 µL of Working Reagent into each Standard and Sample well. Transfer 90 µL of Blank Control Reagent each Sample Blank well. Tap plate to mix.

### Measurement

1. Incubate the plate for 30 minutes at room temperature.
2. Measure the optical density at 570 nm or fluorescence intensity at $\lambda_{Ex}$ = 530 nm/ $\lambda_{Em}$ = 590 nm.

## Results

1. Calculate ΔOD or ΔF by subtracting the blank reading (OD or fluorescence intensity F) of Standard #4 (Blank) from the remaining Standard reading values
2. Plot the ΔF or ΔOD against the standard concentrations.
3. Determine the slope and calculate the creatine concentration of samples using the below equation:

Creatine (µM)=

$$\frac{R_{Sample} - R_{Blank}}{Slope\ (\mu M^{-1})} \times DF$$

Where:
$R_{Sample}$ = OD or fluorescence intensity (F) reading of Sample

$R_{Blank}$ = OD or fluorescence intensity (F) reading of Sample Blank

DF = Sample dilution factor (DF = 1 for undiluted Samples)

**Note:** If the calculated creatine concentration is higher than 1000 µM for the colorimetric assay or 50 µM for the fluorometric assay, dilute the sample in purified water and repeat assay. Multiply result by the dilution factor (DF).

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

**Figure 1.**

Typical Colorimetric Creatine Standard Curve

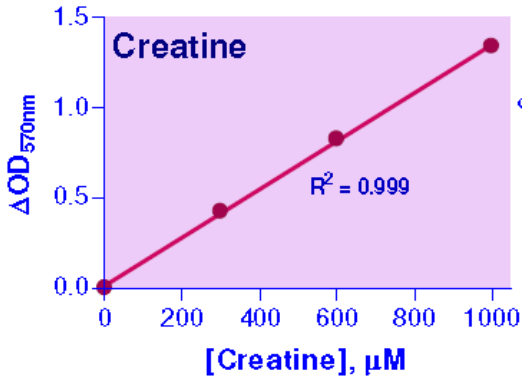
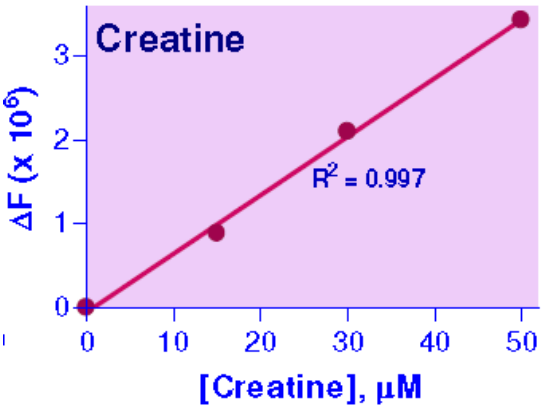



**Figure 2.**

Typical Fluorometric Creatine Standard Curve



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