

## **Technical Manual**

## **ColorFluor Creatine Assay Kit**

**Catalogue Code: BA0095**

**Pack Size: 100 assays**

**Research Use Only**

## 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 Assay Genie ColorFluor 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  $\lambda_{em/ex} = 590/530$  nm is directly proportional to the creatine concentration in the sample.

## KEY FEATURES

**High sensitivity and wide linear range.** Use 10  $\mu$ L sample. Linear detection range 4 to 1000  $\mu$ M (colorimetric) or 0.5 to 50  $\mu$ M (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)

<b>Assay Buffer:</b> 20 mL	<b>Enzyme A:</b> 120 $\mu$ L
<b>Enzyme B:</b> 220 $\mu$ L	<b>Standard:</b> 400 $\mu$ L 20 mM creatine
<b>Dye Reagent:</b> 220 $\mu$ L	

**Storage conditions.** The kit is shipped chilled. Store all components at  $-20^{\circ}\text{C}$ . 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 ( $\text{dH}_2\text{O}$ ) 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  $\mu$ M creatine Standard Premix by mixing 15  $\mu$ L of the 20 mM Standard and 285  $\mu$ L  $\text{dH}_2\text{O}$ . Dilute Standard as follows.

No	Premix + $\text{dH}_2\text{O}$	Vol ( $\mu$ L)	Creatine ( $\mu$ M)
1	100 $\mu$ L + 0 $\mu$ L	100	1000
2	60 $\mu$ L + 40 $\mu$ L	100	600
3	30 $\mu$ L + 70 $\mu$ L	100	300
4	0 $\mu$ L + 100 $\mu$ L	100	0

Transfer 10  $\mu$ L standards into separate wells of a clear, flat-bottom 96-well plate.

Transfer 10  $\mu$ L of each sample into two separate wells, one serving as a sample blank well ( $R_{\text{BLANK}}$ ) and one as a sample well ( $R_{\text{SAMPLE}}$ ).

2. **Enzyme Reaction.** For each standard and sample well, prepare Working Reagent by mixing 90  $\mu\text{L}$  Assay Buffer, 1  $\mu\text{L}$  Enzyme A, 1  $\mu\text{L}$  Enzyme B and 1  $\mu\text{L}$  Dye Reagent. Add 90  $\mu\text{L}$  Working Reagent to the *four Standards* and the *Sample Wells*.

Prepare blank control reagent by mixing 90  $\mu\text{L}$  Assay Buffer, 1  $\mu\text{L}$  Enzyme B and 1  $\mu\text{L}$  Dye Reagent (i.e. no Enzyme A). Add 90  $\mu\text{L}$  Blank control reagent only to the *Sample Blank Wells*.

Tap plate to mix. Incubate 30 min at room temperature.

3. Read  $\text{OD}_{570\text{nm}}$ .

### Fluorimetric Procedure

The fluorimetric procedure is the same as for the colorimetric assay, except that (1) the detection range is up to 50  $\mu\text{M}$  creatine and (2) a black, flat-bottom 96-well plate is used. Creatine standards of 0, 15, 30 and 50  $\mu\text{M}$  are prepared. After incubation for 30 min at room temperature, read fluorescence intensity at  $\lambda_{\text{ex}} = 530 \text{ nm}$  and  $\lambda_{\text{em}} = 590 \text{ nm}$ .

### CALCULATION

Subtract the standard values from the blank value (#4) and plot the  $\Delta\text{OD}$  or  $\Delta\text{F}$  against standard concentrations. Determine the slope and calculate the creatine concentration of Sample,

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

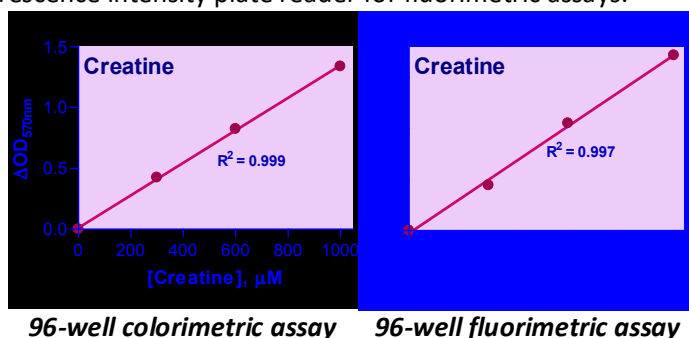
$R_{\text{SAMPLE}}$  and  $R_{\text{BLANK}}$  are optical density or fluorescence intensity readings of the Sample and Sample Blank, respectively.  $n$  is the sample dilution factor.

*Note:* if the calculated creatine concentration is higher than 1000  $\mu\text{M}$  in the colorimetric assay or 50  $\mu\text{M}$  in the fluorimetric assay, dilute sample in  $\text{dH}_2\text{O}$  and repeat assay. Multiply result by the dilution factor  $n$ .

*Conversions:* 1000  $\mu\text{M}$  creatine equals 13.1  $\text{mg/dL}$  or 131  $\text{ppm}$ .

### 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.



### LITERATURE

- Jabs, C.M. et al (1988). Plasma creatine determination using a luminescence method. *Biochem Med Metab Biol.* 39(3):267-272.
- Anderson, D.R. et al (1957). Determination of creatine in biological fluids. *Biochem J.* 67(2): 258-262
- Delanghe, J. et al (1986). Early diagnosis of acute myocardial infarction by enzymatic urinary creatine determination. *Clin Chem.* 32(8):1611.

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