

## EnzyChrom™ Creatinine Assay Kit (EICT-100)

### Quantitative Colorimetric and Fluorimetric Creatinine Determination

#### DESCRIPTION

**CREATININE** is synthesized in the body from creatine, which is produced during muscle contractions from creatine phosphate. In the blood, creatinine is removed by filtration through the glomeruli of the kidney and is secreted into urine. In healthy individuals, creatinine secretion is independent of diet and is fairly constant. The creatinine clearance test has become one of the most sensitive tests for measuring glomerular filtration rate. In kidney disease, creatinine levels in the blood are elevated, whereas the creatinine clearance rate and hence the urine levels are diminished. Creatinine testing is most widely used to assess kidney function.

BioAssay Systems' enzyme-based creatinine assay uses a reaction sequence that excludes both endogenous creatine and ammonia to convert a dye into a colored and fluorescent form. The absorbance at 570 nm or fluorescence intensity at  $\lambda_{\text{ex/em}} = 530/585$  nm is directly proportional to the creatinine concentration in the sample.

#### KEY FEATURES

**Fast and sensitive.** Linear detection range: 10.6 to 500  $\mu\text{M}$  or 0.12-5.7 mg/dL (colorimetric assay) and 0.25 to 50  $\mu\text{M}$  or 0.0028-0.57 mg/dL (fluorimetric assay) for a 60 min reaction.

**Convenient.** The procedure involves adding a single working reagent and reading after 60 minutes.

**High-throughput.** Homogeneous "mix-incubate-measure" type assay. Can be readily automated to process thousands of samples per day.

#### APPLICATIONS

Creatinine determination in urine, serum, plasma, and other biological preparations.

#### KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

<b>Assay Buffer:</b> 10 mL	<b>ATP:</b> 120 $\mu\text{L}$
<b>Enzyme A:</b> 120 $\mu\text{L}$	<b>Dye Reagent:</b> 120 $\mu\text{L}$
<b>Enzyme Mix:</b> 120 $\mu\text{L}$	<b>Standard:</b> 1 mL

**Storage conditions.** The kit is shipped on ice. Store all components at  $-20^{\circ}\text{C}$  upon receiving. Shelf life: 6 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 Safety Data Sheet for detailed information.

#### PROCEDURES

##### Sample Preparation

Urine samples should be diluted at least 100-fold with  $\text{dH}_2\text{O}$  before the assay.

Serum and Plasma samples should be deproteinated using a 10 kDa spin filter (e.g. Corning Cat# 431483) and the filtrate can be assayed directly. Alternatively, the deproteinated serum or plasma can be assayed with an internal standard method (see Product FAQs).

##### Reagent Preparation

Equilibrate all reagents to room temperature before assay. Briefly centrifuge tubes before opening

##### Colorimetric Procedure

1. **Standards.** Prepare 200  $\mu\text{L}$  of 500  $\mu\text{M}$  Premix by mixing 50  $\mu\text{L}$  of the Standard (2 mM) and 150  $\mu\text{L}$   $\text{dH}_2\text{O}$ . Dilute standards in 1.5-mL centrifuge tubes as described in the Table. Transfer 20  $\mu\text{L}$  Standards into separate wells of a *clear, flat bottom 96-well plate*.

No	Premix + $\text{H}_2\text{O}$	Standard ( $\mu\text{M}$ )
1	100 $\mu\text{L}$ + 0 $\mu\text{L}$	500
2	60 $\mu\text{L}$ + 40 $\mu\text{L}$	300
3	30 $\mu\text{L}$ + 70 $\mu\text{L}$	150
4	0 $\mu\text{L}$ + 100 $\mu\text{L}$	0

2. Transfer 20  $\mu\text{L}$  of each sample into separate wells.

- Prepare enough Working Reagent (WR) for all assay wells by mixing, for each well, 80  $\mu\text{L}$  Assay Buffer, 1  $\mu\text{L}$  Enzyme A, 1  $\mu\text{L}$  Enzyme Mix, 1  $\mu\text{L}$  ATP, and 1  $\mu\text{L}$  Dye Reagent.
- Add 80  $\mu\text{L}$  of the WR to each well. Tap plate briefly to mix.
- Incubate at room temperature for 60 min. Use a plate reader to read  $\text{OD}_{570\text{nm}}$ .

##### Fluorimetric Procedure

Dilute the standards prepared in Colorimetric Procedure 1:10 in  $\text{dH}_2\text{O}$ .

Transfer 20  $\mu\text{L}$  standards and 20  $\mu\text{L}$  samples into separate wells of a *black 96-well plate*.

Add 80  $\mu\text{L}$  of the Working Reagent to each well (see Colorimetric Procedure step 3). Tap plate to mix.

Incubate protected from light for 60 min at RT and read fluorescence at  $\lambda_{\text{ex/em}} = 530/585$  nm.

#### CALCULATION

Subtract blank value (water, standard #4) from the standard values and plot the adjusted values against standard concentrations. Determine the slope and calculate the creatinine concentration of Sample as follows

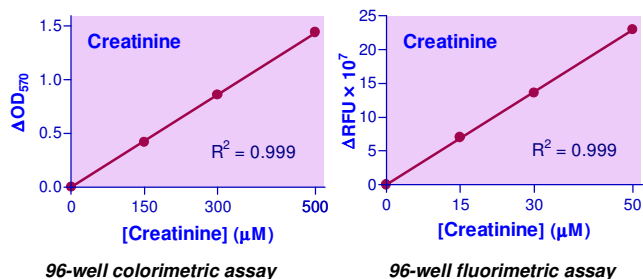
$$[\text{Creatinine}] = \frac{R_{\text{SAMPLE}} - R_{\text{BLANK}}}{\text{Slope } (\mu\text{M}^{-1})} \times n \quad (\mu\text{M})$$

where  $R_{\text{SAMPLE}}$  and  $R_{\text{BLANK}}$  are the OD or fluorescence values of the Sample and  $\text{H}_2\text{O}$  Blank, respectively.  $n$  is the sample dilution factor.

**Conversions:** 1  $\mu\text{M}$  creatinine equals 0.0113 mg/dL.

#### MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories (e.g. multi-channel pipettor, pipette tips, etc), clear (colorimetric) or black (fluorimetric) flat-bottom 96-well plates, centrifuge tubes, and plate reader.



#### LITERATURE

- Perrone, R.D., et al. (1992) Serum creatinine as an index of renal function: new insights into old concepts. Clin Chem, 38:1933-1953.
- Tietz, N.W. (1999) Fundamentals of Clinical Chemistry, 3<sup>rd</sup> Ed. W. B. Saunders Company, pp. 1204-1270.
- Kasiske, B.L., Keane, W.F. (2000) Laboratory assessment of renal disease: clearance, urinalysis, and renal biopsy. The Kidney, 6<sup>th</sup> Ed., WB Saunders Company, pp. 1129-1170.

##### Related Products:

QuantiChrom™ Creatinine Assay Kit (DICT-500)