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 $\lambda_{ex/em}$ = 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 µM or 0.12-5.7 mg/dL (colorimetric assay) and 0.25 to 50 µM 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)

Assay Buffer:	10 mL	**ATP:**	120 µL
Enzyme Mix:	Dried	**Dye Reagent:**	120 µL
Standard:	1 mL		

Storage conditions. The kit is shipped on ice. Store all components at -20°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 dH_2O before the assay.

Serum and plasma samples should be deproteinated by centrifugation for 15 min at 14000 rpm at room temperature through a 10 kDa spin filter (e.g. Corning Cat# 431483). The filtrate can be assayed directly.

Reagent Preparation

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

Colorimetric Procedure

1. *Standards*. Prepare 200 µL of 500 µM Premix by mixing 50 µL of the Standard (2 mM) and 150 µL dH_2O. Dilute standards in 1.5-mL centrifuge tubes as described in the Table. Transfer 20 µL Standards into separate wells of a *clear, flat bottom 96-well plate*.

No	Premix + H_2O	Standard (µM)
1	100 µL + 0 µL	500
2	60 µL + 40 µL	300
3	30 µL + 70 µL	150
4	0 µL + 100 µL	0

2. Transfer 20 µL of each sample into separate wells.
3. Reconstitute Enzyme Mix with 120 µL Assay Buffer. Pipette until all dried enzyme is dissolved. Prepare enough Working Reagent (WR) for all assay wells by mixing, for each well, 80 µL Assay Buffer, 1 µL Enzyme Mix, 1 µL ATP, and 1 µL Dye Reagent.

   Note: Reconstituted Enzyme Mix is stable for at least 1 month when stored at -20°C.
4. Add 80 µL of the WR to each well. Tap plate briefly to mix.
5. Incubate at room temperature for 60 min. Use a plate reader to read OD_{570nm}.

Fluorimetric Procedure

Dilute the standards prepared in Colorimetric Procedure 1:10 in dH_2O.

Transfer 20 µL standards and 20 µL samples into separate wells of a *black 96-well plate*.

Add 80 µL 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 $\lambda_{ex/em}$ = 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

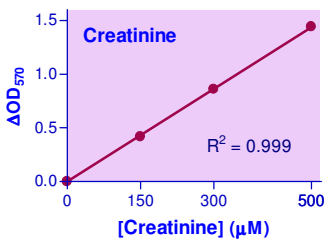
$$[\text{Creatinine}] = \frac{R_{SAMPLE} - R_{BLANK}}{\text{Slope } (\mu M^{-1})} \times n \quad (\mu M)$$

where R_{SAMPLE} and R_{BLANK} are the OD or fluorescence values of the Sample and H_2O Blank, respectively. *n* is the sample dilution factor.

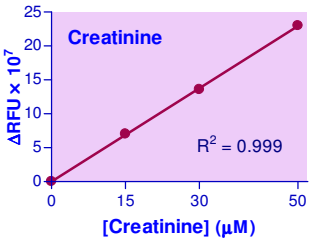
Conversions: 1 µM creatinine equals 0.0113 mg/dL.

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.



96-well colorimetric assay



96-well fluorimetric assay

LITERATURE

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

Related Products:
QuantiChrom™ Creatinine Assay Kit (DICT-500)