QuantiChrom™ Creatinine Assay Kit (DICT-500)

Quantitative Colorimetric Creatinine Determination at 510nm

DESCRIPTION

Creatinine is synthesized in the body at a fairly constant rate 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 test is most widely used to assess kidney function.

Simple, direct and automation-ready procedures for measuring creatinine concentration in biological samples are becoming popular in Research and Drug Discovery. BioAssay Systems' creatinine assay kit is designed to measure creatinine directly in biological samples without any pretreatment. The improved Jaffe method utilizes picrate that forms a red colored complex with creatinine. The intensity of the color, measured at 510nm, is directly proportional to creatinine concentration in the sample. The optimized formulation substantially reduces interference by substances in the raw sample.

KEY FEATURES

Sensitive and accurate. Use 30 µL samples. Linear detection range 0.10 mg/dL (8µM) to 50 mg/dL (4.4mM) creatinine in 96-well plate assay.
Simple and high-throughput. The procedure involves addition of a single working reagent and incubation for 5 min. Can be automated as a high-throughput assay for thousands of samples per day.
Improved reagent stability and versatility. The optimized formulation has greatly enhanced reagent and signal stability. Assays can be executed in 96-well plate or cuvet.
Low interference in biological samples. No pretreatments are needed. Assays can be directly performed on raw biological samples.

APPLICATIONS

Direct Assays: urine, serum, plasma and biological preparations.
Drug Discovery/Pharmacology: effects of drugs on creatinine metabolism.

KIT CONTENTS (500 tests in 96-well plates)

Reagent A: 50 mL Reagent B: 50 mL
Creatinine Standard: 1 mL 50 mg/dL

Storage conditions. All components are stable at 4°C for 12 months.

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

This assay is based on a kinetic Jaffe reaction. To ensure identical incubation time, addition of Working Reagent to standard and samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

Reagent Preparation: equilibrate reagents to room temperature. Prepare working solution by mixing equal volumes of Reagents A and B shortly prior to assay.

Procedure using 96-well plate:

1. Set up standards and samples. Dilute standard to 2 mg/dL by mixing 8 µL standard stock and 192 µL distilled water. Serum and plasma samples can be assayed directly (*n* = 1). Urine should be diluted 50-fold in distilled water prior to assay (*n* = 50). Transfer 30 µL diluted standard and samples in duplicate into wells of a clear bottom 96-well plate. Standard can be stored at 4°C for future use.
2. Using a multi-channel pipettor, add 200 µL working reagent quickly to all standard and sample wells. Tap plate briefly to mix.
3. Incubate at room temperature and read optical density at 1 min (OD_1) and 5 min (OD_5) at 490-530nm (peak absorbance at 510nm).

Procedure using cuvette:

1. Prepare diluted standard and samples as described for 96-well protocol. Transfer 100 µL Standard and samples to cuvets.
2. Add 1000 µL Working Reagent *quickly* to each cuvet and pipet *briefly* to mix (avoid bubble formation).
3. Incubate at room temperature. Read OD for standard and each sample at 1 min (OD_1) and 5 min (OD_5) at 490-530nm (peak 510nm).

CALCULATION

Creatinine concentration of the sample is calculated as

$$= \frac{OD_{SAMPLE\ 5} - OD_{SAMPLE\ 1}}{OD_{STD\ 5} - OD_{STD\ 1}} \times n \times 2 \text{ (mg/dL)}$$

$OD_{SAMPLE\ 5}$ and $OD_{SAMPLE\ 1}$ are OD_{510nm} values of sample at 5 min and 1 min. $OD_{STD\ 5}$ and $OD_{STD\ 1}$ are OD_{510nm} values of standard at 5 min and 1 min, respectively. *n* is the dilution factor. The second reading can be 5 min up to 10 min, as the kinetics is linear within 10 min.
Conversions: 1 mg/dL creatinine equals 88.4 µM, 0.001% or 10 ppm.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting devices and accessories (e.g. multi-channel pipettor).

Procedure using 96-well plate:

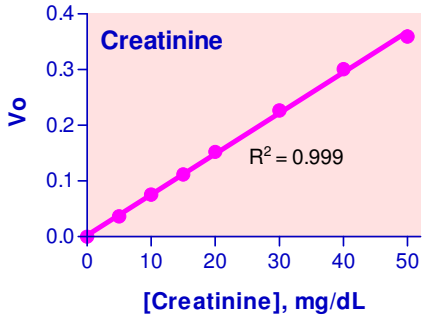
Clear bottom 96-well plates (e.g. Corning Costar) and plate reader.

Procedure using cuvette:

Spectrophotometer and cuvets for measuring OD 510nm.

EXAMPLES

Samples were assayed in duplicate (n = 2) using the 96-well plate protocol. The creatinine concentration (mg/dL) was 0.38 ± 0.01 for rat serum, 0.71 ± 0.02 for rat plasma, 0.79 ± 0.00 for human serum, 0.89 ± 0.04 for human plasma, 1.20 ± 0.04 for goat serum and 136.4 ± 2.9 in a fresh human urine sample.



Calibration curve in 96-well plate
V_o is the initial rate (OD_5 - OD_1)/4 of the reaction

LITERATURE

1. Walser M. (1998) Assessing renal function from creatinine measurements in adults with chronic renal failure. Am J Kidney Dis 32: 23-31.

2. Rajs G and Mayer M (1992). Oxidation markedly reduces bilirubin interference in the Jaffe creatinine assay. Clin Chem. 38: 2411-2413.

3. Cook JG (1971). Creatinine assay in the presence of protein. Clin Chim Acta. 32: 485-486.