CREATIVE BIOMART®
Assay Kit

Creatinine Assay Kit

Product Information

Cat.No. Kit-0261

Product Overview

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.

Applications

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

Storage

The kit is shipped at room temperature. Store Reagents A and B at 2-8°C and Creatinine Standard at -20°C. Shelf life: 12 months after receipt.

Size

500 tests

Kit Components

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

Materials Required but Not Supplied

Pipeting devices and accessories (e.g. multi-channel pipettor). Clear bottom 96-well plates (e.g. Corning Costar) and plate reader for the plate procedure. Spectrophotometer and cuvets for measuring OD 510nm for the cuvette procedure.

Detection method OD510nm

Features & Benefits

Tel: 1-631-559-9269 1-516-512-3133
Email:info@creative-biomart.org
Fax:1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

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Sensitive and accurate. Use 30 µL samples. Detection limit 0.10 mg/dL (8µM) 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.

Assay Protocol

Equilibrate reagents to room temperature prior to use. Please note the difference in standard/sample volume and Working Reagent strength for blood and urine assays. 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.
Procedure using 96-well plate:
BLOOD ASSAY (LOW CREATININE LINEAR UP TO 50 mg/dL):
1. Dilute standard to 2 mg/dL by mixing 5 µL 50 mg/dL standard stock and 120 µL distilled water. Transfer 30 µL diluted standard and serum/plasma in duplicate into wells of a clear bottom 96-well plate.
2. Prepare enough Working Reagent by mixing per well reaction at least 100 µL Reagent A and 100 µL Reagent B. Add 200 µL Working Reagent quickly to all wells. Tap plate briefly to mix.
3. Read optical density immediately (OD0) and then at 5 min (OD5) at 490-530nm (peak absorbance at 510nm).

URINE ASSAY (HIGH CREATININE LINEAR UP TO 300 mg/dL):
1. Transfer 5 µL 50 mg/dL standard and urine in duplicate into wells of a clear bottom 96-well plate.
2. Prepare enough Working Reagent by mixing per well reaction 50 µL Reagent A, 50 µL Reagent B and 100 µL water. Add 200 µL Working Reagent quickly to all wells. Tap plate briefly to mix.
3. Read optical density immediately (OD0) and then at 5 min (OD5) at 490-530nm (peak

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absorbance at 510nm).

Procedure using cuvette:
1. Transfer 100 µL 2 mg/dL Standard and serum/plasma samples (Urine Assay: 15 µL 50 mg/dL Standard and 15 µL urine) to cuvets.
2. Prepare appropriate Working Reagent as above for the 96-well plate procedures. Add 1000 µL Working Reagent to each cuvet and pipet briefly to mix (avoid bubble formation).
3. Read OD immediately (OD0) and at 5 min (OD5) at 490-530nm.

Analysis

Creatinine concentration of the sample is calculated as
= (ODSAMPLE 5 – ODSAMPLE0)/(ODSTD 5 – ODSTD 0)x [STD] (mg/dL)
ODSAMPLE5, ODSAMPLE0, ODSTD5 and ODSTD0 are OD510nm values of sample and standard at 5 and 0 min, respectively. [STD] is 2 mg/dL for blood assay and 50 mg/dL for urine assay.
Conversions: 1 mg/dL creatinine equals 88.4 µM, 0.001% or 10 ppm.

Sensitivity

0.1 mg/dL (8 µM)