Creatinine Assay Kit

Creatinine Assay Kit is a detection kit for the quantification of Creatinine in serum, plasma and urine.

Catalog number: ARG82121

Package: 500 tests

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INTRODUCTION

Creatinine is a breakdown product of creatine phosphate from muscle and protein metabolism. It is released at a constant rate by the body (depending on muscle mass).

Serum creatinine (a blood measurement) is an important indicator of kidney health because it is an easily measured by product of muscle metabolism that is excreted unchanged by the kidneys. Creatinine itself is produced via a biological system involving creatine, phosphocreatine (also known as creatine phosphate), and adenosine triphosphate (ATP, the body's immediate energy supply). [Provide by Wikipedia: Creatinine]

PRINCIPLE OF THE ASSAY

This Creatinine Assay Kit is a simple colorimetric assay that measures the amount of Creatinine present in serum, plasma and urine. The assay 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 O.D. 510 nm, is directly proportional to creatinine concentration in the sample.

MATERIALS PROVIDED & STORAGE INFORMATION

Store Reagents A and B at 2-8°C and Creatinine Standard at -20°C. Shelf life: 12 months after receipt.

Component	Quantity	Storage information
Reagent A	50 mL	4°C
Reagent B	50 mL	4°C
Creatinine Standard (50 mg/dL)	1 mL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 510 nm
- Centrifuge
- Clear flat-bottom 96 well plate
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- 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.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes at 4°C.

Plasma: Collect blood with EDTA, heparin or citrate and centrifuge at 2000 x g for 10 minutes at 4°C. Collect the plasma layer and store on ice.

Urine: Use directly.

Note:

1. All samples can be stored at-20 to -80°C for at least one month.

ASSAY PROCEDURE

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 for serum and plasma (LOW CREATININE LINEAR UP TO 50 mg/dL):

1. Dilute standard to 2 mg/dL by mixing **5 µL** of **50 mg/dL Standard stock** and **120 µL** of **distilled water**. Transfer **30 µL** of **diluted standard** and **samples** in duplicate into wells of a **clear bottom 96-well microplate**.
2. Prepare enough **Working Reagent** by mixing per well reaction at least **100 µL** of **Reagent A** and **100 µL** of **Reagent B**. Add **200 µL** of **Working Reagent** quickly to all wells. Tap plate briefly to mix.
3. Read the absorbance at **O.D. 490-530 nm (peak absorbance at 510nm) immediately** (OD_0) and then at **5 min** (OD_5).

Procedure for urine (HIGH CREATININE LINEAR UP TO 300 mg/dL):

1. Transfer **5 µL** of **50 mg/dL Standard** and **urine sample** in duplicate into wells of a **clear bottom 96-well microplate**.
2. Prepare enough **Working Reagent** by mixing per well **50 µL** of **Reagent A**, **50 µL** of **Reagent B** and **100 µL** of **distilled water**. Add **200 µL** of **Working Reagent** quickly to all wells. Tap plate briefly to mix.
3. Read the absorbance at **O.D. 490-530 nm (peak absorbance at 510nm) immediately** (OD_0) and then at **5 min** (OD_5).

CALCULATION OF RESULTS

1. Creatinine concentration of the sample is calculated as:

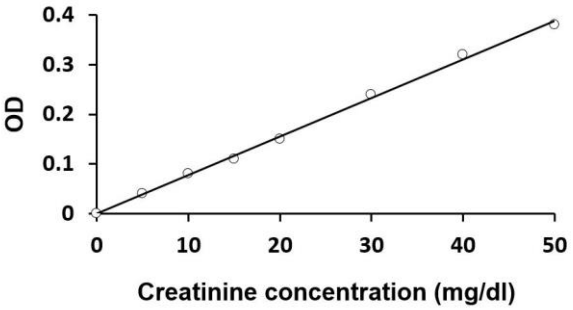
   Creatinine (mg/dL) = [($OD_{Sample5}$ – $OD_{Sample0}$) / (OD_{STD5} – OD_{STD0})] x [STD]

   Note:

   - $OD_{Sample5}$ / ODS_{ample0}: value of sample at 5 minutes and 0 minutes.
   - OD_{STD5} / OD_{STD0}: value of Standard at 5 minutes and 0 minutes.
   - [STD]: 2 mg/dL for blood sample; 50 mg/dL for urine sample.
   - Conversions: 1 mg/dL creatinine = 88.4 µM = 0.001% = 10 ppm.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Creatinine Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

0.1 mg/dL (8 µM)