Creatinine Assay Kit

Catalog No.: abx298944

Size: 96 tests

Detection Range: 20.45 µmol/L – 800 µmol/L

Sensitivity: 3.8 µmol/L

Storage: Store all components at 4°C for up to 12 months.

Application: For detection and quantification of Creatinine content in serum, plasma, urine and other biological fluids.

Introduction

Creatinine is a metabolite formed from creatine and phosphocreatine. Creatine and phosphocreatine are converted to creatinine in a non-enzymatic fashion, which then enters the circulatory system. Under basal physiological conditions, creatinine is filtered by the glomerulus and excreted by the kidneys. Creatine is predominantly located in the heart, brain and muscles.

Abbexa's Creatinine Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Creatinine content. Creatinine can be catalyzed into creatine, which in turn is broken down into sarcosine and urea by the enzyme creatinase. Sarcosine oxidase acts on sarcosine to form hydrogen peroxide, which reacts with 4-ampyrone to form a pink compound with an absorbance peak at 515 nm. The intensity of the color at 515 nm is proportional to the activity of the Creatinine content, which can then be calculated.

Kit components

1. 96-well microplate
2. Enzyme solution A: 20 ml
3. Enzyme solution B: 7 ml
4. Standard solution (1000 µmol/L): 2 x 1.5 ml
5. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (515 nm)
2. Distilled water
3. Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. Vials/tubes
6. Sonicating water bath
7. Centrifuge
8. Vortex mixer

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum:** Allow fresh blood to stand in a separator tube at 25°C for up to 1 hour, until it clots. Centrifuge at approximately 2000 × g for 15 mins at 4°C. If a precipitate appears, centrifuge again. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Plasma:** Collect plasma using heparin as the anticoagulant. Centrifuge at 1000 – 2000 x g for 10 minutes at 4°C, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples. Take the supernatant (avoid taking the middle layer containing white blood cells and platelets), keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Urine:** Collect fresh urine and centrifuge at 10,000 x g for 15 minutes at 4°C. Collect the supernatant and keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.

Samples should not contain detergents such as SDS Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with normal saline (0.9% NaCl), then carry out the assay procedure. The concentrations of each sample must fall within the kit's stated range (20.45 µmol/L – 800 µmol/L).

The recommended dilution factor for Human, Rat, and Porcine serum is 1. The recommended dilution factor for Human urine is 40 – 60, depending on the sample source.

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. **Standard Dilutions:** Label 7 tubes with 400 µmol/L, 350 µmol/L, 300 µmol/L, 250 µmol/L, 200 µmol/L, 150 µmol/L, and 100 µmol/L. Add 80 µl, 70 µl, 60 µl, 50 µl, 40 µl, 30 µl, and 20 µl of Standard (1 mmol/L) to the 400 µmol/L, 350 µmol/L, 300 µmol/L, 250 µmol/L, 200 µmol/L, 150 µmol/L, and 100 µmol/L tubes respectively, followed by 120 µl, 130 µl, 140 µl, 150 µl, 160 µl, 170 µl, and 180 µl of Distilled water, to prepare the Standard Solutions with concentrations 400 µmol/L, 350 µmol/L, 300 µmol/L, 250 µmol/L, 200 µmol/L, 150 µmol/L, and 100 µmol/L. These volumes are summarized in the following table:

Standard Dilution (µmol/L)	**400**	**350**	**300**	**250**	**200**	**150**	**100**
1 mmol/L Standard (µl)	80	70	60	50	40	30	20
Distilled water (µl)	120	130	140	150	160	170	180

For the blank, or 0 µmol/ml standard, use pure Distilled water. Label an eighth tube as 0 µmol/L, and add 200 µl Distilled water. The volume of each standard will be 200 µl.

B. Assay Procedure

Enzymatic Reaction:

1. Set the sample, standard and blank wells. *It is recommended to run each of these in duplicate*.
2. Add 12 µl of freshly prepared standard solution to each well.
3. Add 12 µl of sample to each sample well.
4. Add 180 µl of Enzyme Solution A into all wells.
5. Incubate in a water bath at 37°C for 5 minutes.
6. Add 60 µl of Enzyme Solution B into all wells.
7. Incubate at 37°C in a water bath for 2 minutes and measure the OD value (A1) of each well at 515 nm.
8. Incubate at 37°C in a water bath for 8 minutes and measure the OD value (A2) of each well at 515 nm.
9. Calculate ΔA = A2 – A1.

C. Calculation of Results

Plot the standard curve, using the OD of the standard dilutions on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = mx + b$. Based on this curve, the concentration of Creatinine in each sample well can be derived with the formula:

$$\text{Creatinine (µmol/L)} = \frac{\Delta A_{515} - b}{a} \times 1000 \times f$$

where:

ΔA_{515}	OD_{Sample} - OD_{Blank}
a	gradient of the standard curve (linear fit)
b	y-intercept of the standard curve (linear fit)
f	dilution factor of the sample before the test