Catalog# 80350

V.4

Crystal Chem

Mouse Creatinine Kit Instructions

For the quantitative determination of creatinine
in mouse serum and urine

Catalog #80350
96 Assays

For research use only. Not for use in diagnostic procedures.

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A. Intended Use

The Mouse Creatinine kit is for the quantitative determination of creatinine in mouse serum and urine. Please read the complete kit insert before performing this assay. The kit is for RESEARCH USE ONLY. It is not intended for use in diagnostic procedures.

B. Introduction

Creatinine is a chemical waste molecule that is produced from creatine. Creatinine is chiefly filtered out of the blood by the kidneys and is disposed of through urine. The kidneys maintain creatinine in a normal range. If the kidneys are not functioning properly, creatinine blood and urine levels rise and can be used to identify abnormal kidney function.

C. Principle of the Assay

The Mouse Creatinine kit is a quick, easy to use enzymatic procedure. The enzymatic assay for mouse creatinine involves a series of enzymatic reactions including enzymatic conversion of creatinine into creatine which is then converted to sarcosine. This is followed by the oxidation of sarcosine to produce hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide can then be quantified at 550 nm and a quantitative determination of creatinine can be made.

D. Kit Storage

1. Upon receipt of the Mouse Creatinine kit, store it at 2-8°C and avoid light exposure (do not freeze the kit or hold it at temperatures above 25°C).
2. The kit should not be used after the expiration date.

E. Assay Materials

E.1. Materials provided

TABLE 1 Contents of the kit

Mark	Description	Amount
CC1	Reagent CC1 (liquid)	1 X 30 mL
CC2	Reagent CC2 (liquid)	1 X 10 mL
CAL1	Creatinine Calibrator 1 (liquid)	1 X 1 mL

E.2. Materials required but not provided

Large capacity microplates (> 390 µL well volume)
Micropipettes and disposable tips
Clean glass tubes and test tube racks
Volumetric flasks
Incubator (37ºC)
Microplate reader or spectrophotometer or analyzer (should read A_{550} values)
0.9% Saline

F. Assay Precautions

1. Only appropriately-trained personnel should use the kit. Laboratory personnel should wear suitable protective clothing. All chemicals and reagents should be considered potentially hazardous. Avoid ingestion and contact with skin or mucous membranes.
2. Some assay components may contain human sourced materials. Accordingly, all assay components should be handled as if potentially infectious using safe laboratory procedures.
3. Do not use the reagents after the expiration date.
4. Reagents CC1 and CC2 contain < 0.1% sodium azide (NaN_3) as a preservative, which may react with lead and copper plumbing to form highly explosive metal azide. On disposal, flush with a large volume of water to prevent azide buildup.
5. Reagents are light-sensitive. Store in a dark place. Do not let bottles remain open. Keep containers tightly stoppered.

G. Maximizing Kit Performance

1. Given the small sample volumes required (8 µL), pipetting should be done as carefully as possible. A high quality 10 µL or better precision pipette should be used for such volumes. Drops of liquid adhering to the outside of the pipette tips should be removed by wiping to ensure the highest degree of accuracy.
2. In order to prevent the microplate wells from drying out and to get the best results, samples and reagents should be dispensed quickly into the wells.
3. Each calibrator and sample should be assayed in duplicate.
4. The same sequence of pipetting and other operations should be maintained in all procedures.
5. Do not mix reagents that have different lot numbers.

H. Sample Collection

Serum: Collect blood, allow to clot, and centrifuge for 20 min at 2,000 x *g*.

Urine: Collect urine sample from mouse.

It is recommended that samples be used within 1 week of collection when stored refrigerated. If assay is to be performed more than 1 week after collection, samples should be frozen.

I. Assay Procedure

I.1. Preparation of reagents

All reagents are provided ready-to-use and should be brought to room temperature for at least 30 minutes prior to use. Reagents should be stored at 2-8°C immediately after use. Before use, mix the reagents thoroughly by gentle agitation or swirling.

I.2. Preparation of samples, calibrators, and controls

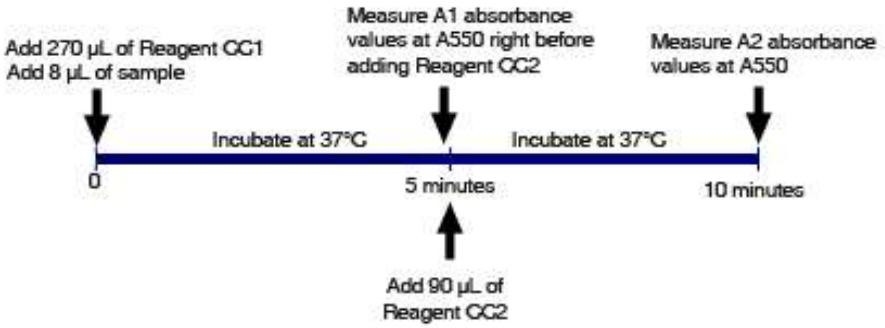
1. Bring all samples, calibrators, and controls to room temperature. Frozen samples should be allowed to fully thaw before proceeding.
   ***Note**: In addition to running the calibrator provided, the assay requires running a blank calibrator. 0.9% saline should be used for running the blank calibrator. Optional controls are sold separately (Cat# 80353).*
2. Prior to running the assay, urine samples should be 10-fold diluted with saline.

I.3. Assay procedure

The procedure below reflects a manual procedure performed using a large capacity microplate (> 390 µL well volume) and a microplate reader (ideal when running multiple samples manually). The procedure can be easily adopted as needed to be run in a glass tube with a spectrophotometer. The assay can also be adopted to work on various automated analyzers using the schema presented in Figure 1. Please contact Crystal Chem for instrument-specific information.

1. Add 270 µL of Reagent CC1 and 8 µL of sample, calibrator, or control into each well (as needed) of a microplate and mix well by repeated pipetting.
2. Place microplate in incubator (37ºC) and allow microplate to equilibrate to 37ºC over 5 minutes.
3. Measure absorbance using a plate reader (measure A_{550} values).
   Note: The Mouse Creatinine assay is an end-point assay and the first reading point A1 is right before the addition of reagent CC2.
4. Add 90 µL of Reagent CC2 and mix well by repeated pipetting.
5. Measure the increase in absorbance after 5 minutes at 37ºC using a plate reader (measure A_{550} values).

Figure 1. Summary of assay procedure



I.4. Determining the mouse creatinine concentration

1. Calculate the change in absorbance ΔA (0sec ~ 300sec)

   $\Delta A = (OD_{550nm,\ 300sec}) - (OD_{550nm,\ 0sec})$

2. Using linear graph paper, construct the creatinine calibration curve by plotting the mean change in absorbance value for the calibrator (incl. blank) on the Y axis versus the corresponding creatinine concentration on the X axis.
   Note: *Calibrator values vary per lot and should be obtained from the calibrator label. A calibration curve should be plotted every time the assay is performed.*

3. Mouse creatinine concentrations in the samples are interpolated using the calibration curve and mean change in absorbance values for each sample. For urine samples, the values obtained must be multiplied by the dilution factor (ie. 10) to obtain the final creatinine concentration. The creatinine concentration is expressed in mg/dL. This interpolation can be simplified using Equation 1 below. The unit of measure can be converted to µmole/L by multiplying the obtained concentration in mg/dL by 88.4.
   Note: *Samples with high mouse creatinine concentrations (13.5 mg/dL or higher in serum or 141 mg/dL or higher in urine) should be diluted with the 0.9% saline and rerun.*

Equation 1. Calculation of mouse creatinine concentration

Mouse creatinine concentration =

[(sample A550 – blank A550) / (calibrator A550 – blank A550)] × calibrator conc.

J. Performance characteristics

J.1. Assay range

The Mouse Creatinine assay has a linear range from 0.15 – 13.5 mg/dL in serum and 0.15 – 141 mg/dL in urine.

J.2. Precision

The assay has a within-run and total precision of CV < 10%.

Warranty

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