# RayBio® Creatinine Assay

For the quantitative determination of Creatinine in urine samples

Catalog #: MA-CTN

User Manual
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Caution:
Extraordinarily useful information enclosed

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Empowering your proteomics

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# Table of Contents



Please read the entire manual carefully before starting your experiment

# I. Introduction

Creatinine is a metabolite waste product of phospho-Creatine (p-Creatine), a molecule used as a store for high-energy phosphate that can be utilized by muscles and other tissues for the production of ATP. Dietary intake and de novo synthesis from the amino acids arginine, glycine, and methionine in the kidney and liver are the primary sources of Creatine. Creatine and p-Creatine are converted non-enzymatically to the metabolite Creatinine, which diffuses into the blood, is filtered through the kidneys, and is then secreted into urine. In vivo, this conversion appears to be irreversible and in vitro it is favored by higher temperatures and lower pH. Some Creatinine may form via p-Creatinine as well. Under normal conditions, Creatinine's formation occurs at a rate that is relatively constant. For instance, in humans approximately 2% of the Creatine/p-Creatine pool is converted to Creatinine daily. This predictability makes Creatinine a useful tool for normalizing the levels of other molecules found in urine. In addition, altered Creatinine levels can be used as an indicator of kidney dysfunction, or may be associated with other conditions that result in decreased renal blood flow. Some examples include diabetes and cardiovascular disease. The RayBio® Creatinine assay is a 30 minute chemical analysis designed to measure Creatinine in urine.

# II. General Description

The RayBio Creatinine Assay in an in vitro quantitative assay for the detection of creatinine based on the Jaffe reaction. In this method, creatinine is treated with an alkaline picrate solution to yield a bright orange-red complex. Diluted samples are added to a microplate, and an alkaline picrate reagent is added and incubated at room temperature for 30 minutes. The intensity of the color at 490 nm corresponds to the concentration of creatinine in the sample. Unknown samples are compared to the standard curve.

# III. Storage

The entire kit should be stored at 2-8 °C for up to 6 months from the date of shipment. Do not use past kit expiration date. For prepared reagent storage, see table below.

# IV.Reagents

| **Component** | **Size / Description** | **Storage / Stability <u>After Preparation</u>** |
|---|---|---|
| Microplate (Item A) | Two 96 wells (12 strips x 8 wells) plates | Store at room temperature* |
| Creatinine Standard | 2.0 mL of Creatinine solution at 100 mg/dL. | 6 months at 2-8°C |
| Picric Acid Reagent | 25 mL of a 0.13% picric acid solution. May contain a precipitate. Heat at 37 °C for 10 minutes and mix to dissolve before adding the sodium hydroxide | Store at room temperature. |
| NaOH | 5.0 mL of 1 N sodium hydroxide | Store at room temperature. |

*Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

# V. Additional Materials Required

1. Microplate reader capable of measuring absorbance at 490 nm
2. Precision pipettes to deliver 2 µl to 1 ml volumes
3. Distilled or deionized water
4. Tubes to prepare standard or sample dilutions

# VI. Reagent Preparation

Keep kit reagents on ice during reagent preparation steps.

## A. **Alkaline Picrate Solution**

1. Add 2.5 mL of NaOH to 12.5 mL of Picric Acid Reagent to prepare the Alkaline Picrate Solution. Mix well.

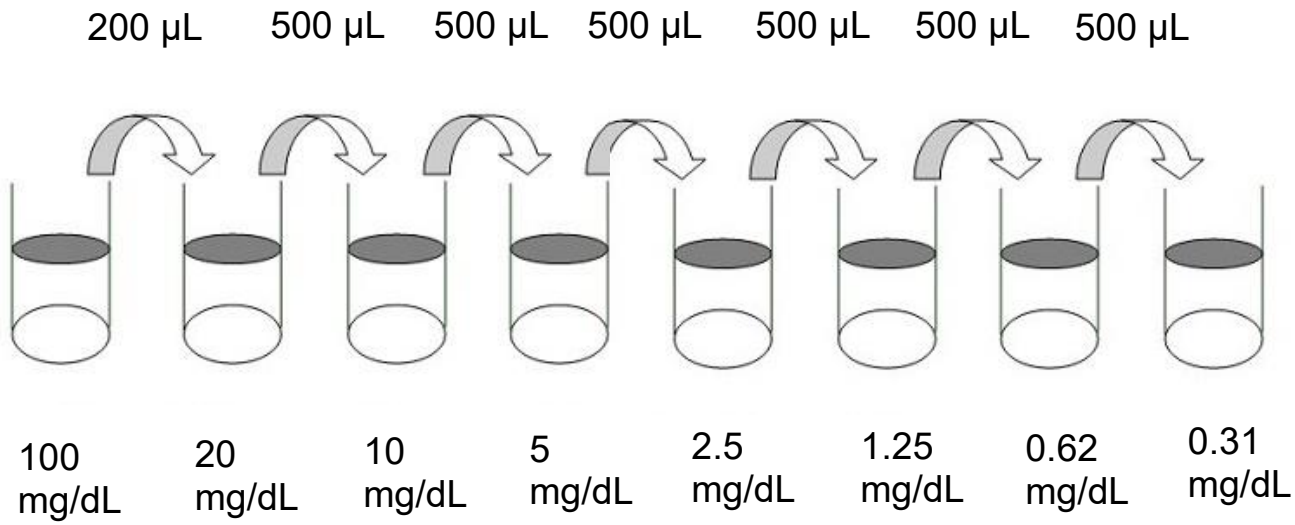
   *Note: Solution may darken and precipitate over time. The performance of the Alkaline Picrate Solution will not be affected by these changes. Precipitate can be dissolved by warming to 37°C for approximately 10 minutes. Mix well to re-dissolve.*

## B. Preparation of Standards

1. Label 8 microtubes with the following concentrations: 20 mg/dL, 10 mg/dL, 5 mg/dL, 2.5 mg/dL, 1.25 mg/dL, 0.62 mg/dL, 0.31 mg/dL, and 0 mg/dL. Pipette 800 µL of distilled or deionized water into the 20 mg/dL tube. Pipette 500 µL of distilled or deionized water into the remaining tubes.

2. Pipette 200 µL of the Creatinine Standard into the 20 mg/dL tube. Mix thoroughly.

3. Repeat this step with each successive concentration, preparing a dilution series as shown in the illustration below. Each time, use 500 µL of the prior concentration until the 0.31 mg/dL is reached. Mix each tube thoroughly before the next transfer. Use deionized or distilled water as the 0 mg/dL standard.



### C. Sample Preparation

1. Urine should be aseptically collected from the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter. Use immediately or aliquot and store at -20$^{o}$C until use. Avoid repeated freeze-thaws.

2. The recommended dilution for urine is 20x with deionized or distilled water.

   Note: Optimal sample dilution factors should be determined empirically. If you have any questions regarding the recommended dilutions you may contact technical support at 888-494-8555 or techsupport@raybiotech.com.

## VII. Assay Procedure

1. Bring all reagents and samples to room temperature before use. It is recommended that all standards and samples be run at least in duplicate.

2. Add 50 µL standard (See Reagent Preparation Section B) and sample (see Reagent Preparation Section C) to appropriate wells.

3. Add 100 µL Alkaline Picrate Solution (see Reagent Preparation Section A) to each well. Incubate for 30 minutes at room temperature.

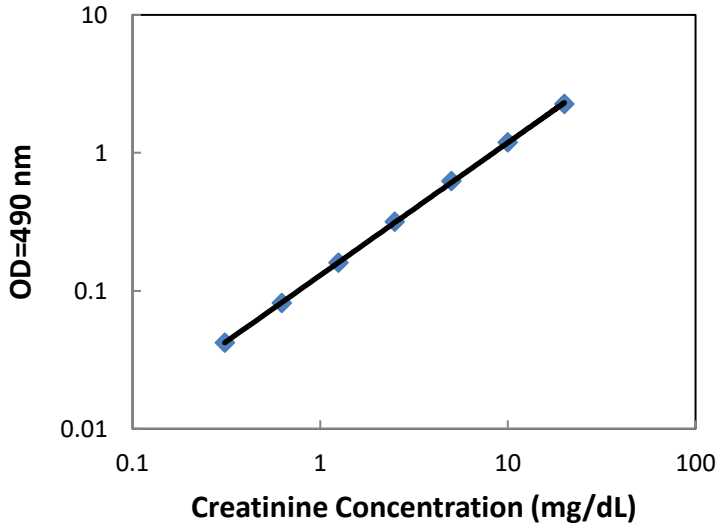
4. Read at 490 nm immediately.

# VIII. Calculation of Results

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

### A. Typical Data

These standard curves are for demonstration only. A standard curve must be run with each assay.




### B. Sensitivity

The minimum detectable concentration of Creatinine is 0.019 mg/dL.
Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank.

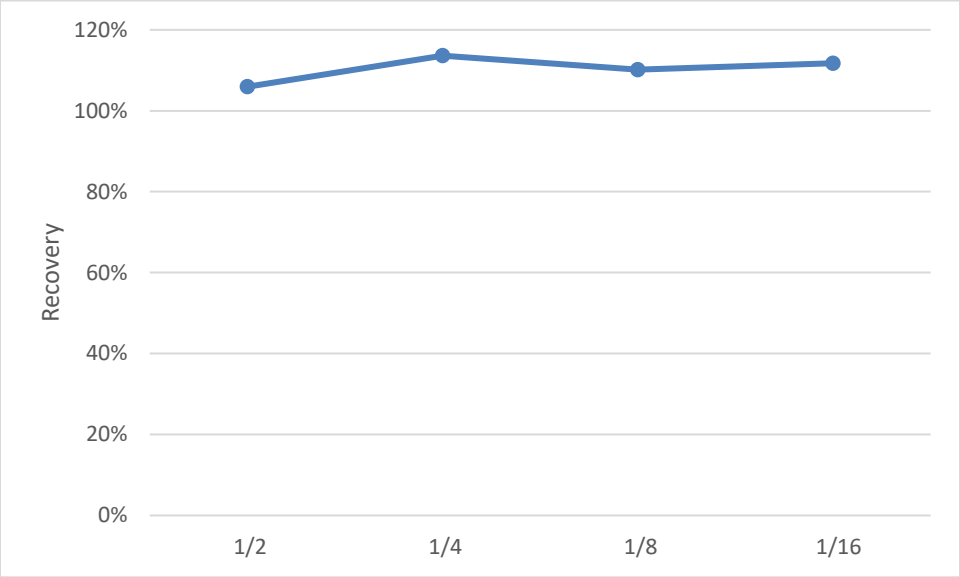
### C. SPIKING & RECOVERY

The recovery of Creatinine spiked to levels throughout the range of the assay was evaluated. Samples were diluted prior to assay.

| Sample Type | Average % Recovery | Range % |
|---|---|---|
| Urine (n=4) | 102 | 99-106 |

## D. LINEARITY

To assess the linearity of the assay, samples containing high concentrations of Creatinine were serially diluted (1:2, 1:4, 1:8, 1:16) to produce samples with values within the dynamic range of the assay.




## E. Reproducibility

Intra-assay Precision (Precision within an assay)

Four samples were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Four samples were tested in forty separate assays to assess inter-assay precision.

| | Intra-Assay Precision | | | | Inter-Assay Precision | | | |
|---|---|---|---|---|---|---|---|---|
| **Sample** | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| **n** | 20 | 20 | 20 | 20 | 40 | 40 | 40 | 40 |
| **Mean** | 0.84 | 0.59 | 0.70 | 0.91 | 0.84 | 0.57 | 0.68 | 0.88 |
| **Standard Deviation** | 0.016 | 0.019 | 0.023 | 0.020 | 0.023 | 0.027 | 0.030 | 0.037 |
| **CV%** | 1.95 | 3.17 | 3.28 | 2.15 | 2.75 | 4.68 | 4.38 | 4.14 |

# IX. Specificity

The RayBio Creatinine assay is designed to measure Creatinine in urine.

# RayBio® ELISA Kits

Over 3,000 ELISA kits available, visit www.RayBiotech.com/ELISA-Kits.html for details.

This product is for research use only.