Parameter™

Creatinine Assay

Catalog Number KGE005

For the quantitative determination of Creatinine in urine samples.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Creatinine is a metabolite of phospho-Creatine (p-Creatine), a molecule used as a store for high-energy phosphate that can be utilized by tissues for the production of ATP (1). The source of Creatine may vary between species. Dietary intake may be accompanied by *de novo* synthesis from the amino acids arginine, glycine, and methionine. This occurs in stepwise fashion primarily in the kidneys and liver, although other organ systems may be involved and species-specific differences may exist (2). The vast majority of Creatine (> 90%) is found in muscle, as well as other tissues including heart, brain, photo-receptors, and testes (2-7).

Creatine and p-Creatine are converted non-enzymatically to the metabolite Creatinine (Figure 1), which diffuses into the blood and is excreted by the kidneys. *In vivo*, this conversion appears to be irreversible and *in vitro* it is favored by higher temperatures and lower pH (2). Some Creatinine may form via p-Creatinine as well (8). 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 (2). This predictability makes Creatinine a useful tool for normalizing the levels of other molecules found in urine (9, 10). 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 (11). Some examples include diabetes and cardiovascular disease (12, 13).

The Parameter Creatinine assay is a 30 minute chemical analysis designed to measure Creatinine in urine.

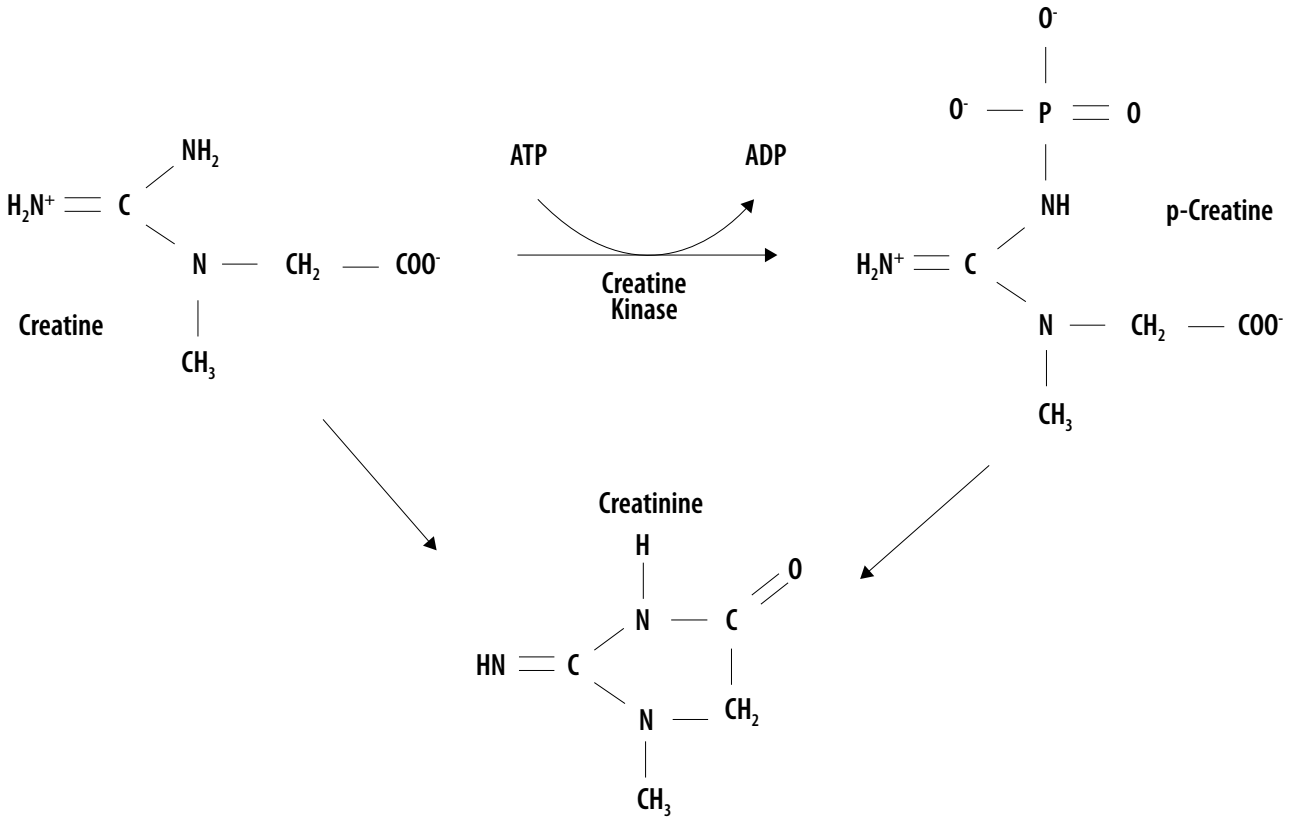


Figure 1: Creatine is converted to p-Creatine via the enzyme Creatine Kinase. Creatinine is formed by the non-enzymatic metabolism of Creatine and p-Creatine.

PRINCIPLE OF THE ASSAY

In the Jaffe reaction, creatinine is treated with an alkaline picrate solution to yield a bright orange-red complex. Diluted samples are added to a microplate, and alkaline picrate reagent is added and incubated at room temperature for 30 minutes. Intensity of the color at 490 nm corresponds to the concentration of creatinine in the sample. Unknown samples are compared to the standard curve.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute with deionized or distilled water and repeat the assay.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is intended for use in urine only, where Creatinine is the primary component that will yield an orange-red color. Other biological components may interfere in the assay (14, 15).
- Some researchers have modified this reaction for use in serum or plasma (16, 17).

PRECAUTIONS

Several of the kit components are hazardous. Sodium hydroxide is corrosive. Picric acid is an irritant and, if dried, potentially explosive. Avoid contact with metals and use large volumes of water during disposal. Take precautions when handling these reagents.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/RECONSTITUTED MATERIAL
Microplates	892880	Two 96 well microplates (12 strips of 8 wells).	Return unused wells to the foil pouch and reseal along entire edge of zip-seal. Store at room temperature.
Creatinine Standard	892890	2.0 mL of Creatinine solution at 100 mg/dL.	May be stored for up to 1 month at 2-8 °C.*
Picric Acid Reagent	892891	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	891236	5.0 mL of 1 N sodium hydroxide.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 490 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Test tubes for dilution of standards and samples.
- Creatinine Controls (optional; commercially available).

SAMPLE COLLECTION & STORAGE

The sample collection and storage condition listed below is intended as a general guideline. Sample stability has not been evaluated.

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Urine samples require 20-fold dilution. A suggested 20-fold dilution is 10 µL of sample + 190 µL of deionized or distilled water.

REAGENT PREPARATION

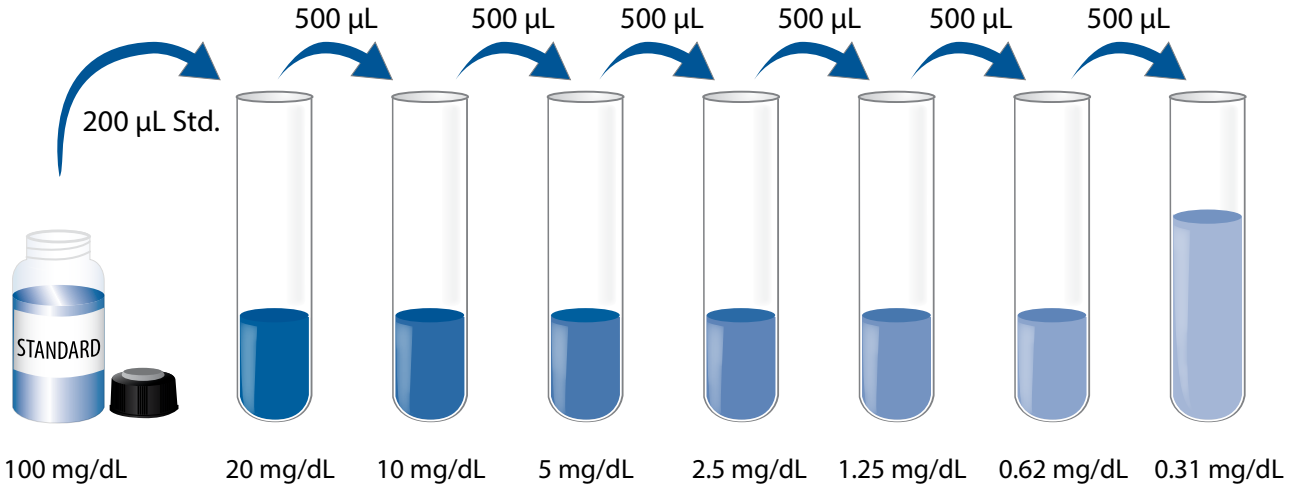
Alkaline Picrate Solution - For one plate add 2.5 mL of NaOH to 12.5 mL of Picric Acid Reagent. Mix well. 100 µL of Alkaline Picrate Solution is required per well.

For two plates, the entire contents of the NaOH bottle may be added to the Picric Acid Reagent bottle.

Note: *Store at room temperature for up to 1 month provided it is within the expiration date of the kit. Solution may darken and precipitate over time. Precipitate can be dissolved by warming to 37 °C for approximately 10 minutes. Mix well to re-dissolve. The performance of the the Alkaline Picrate Solution will not be affected by these changes.*

STANDARD CURVE PREPARATION

Pipette 800 µL of deionized or distilled water into the 20 mg/dL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 20 mg/dL standard serves as the high standard. Use deionized or distilled water as the zero standard (0 mg/dL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, controls, and standards be assayed in duplicate.

1. Prepare all reagents, samples, and Creatinine Standard as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch, and reseal.
3. Add 50 µL Standard, control, or sample* to each well. A plate layout is provided as a record of samples and standards assayed.
4. Add 100 µL Alkaline Picrate Solution to each well. Incubate for 30 ± 5 minutes at room temperature.
5. Determine the optical density of each well using a microplate reader set to 490 nm.

*Samples require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

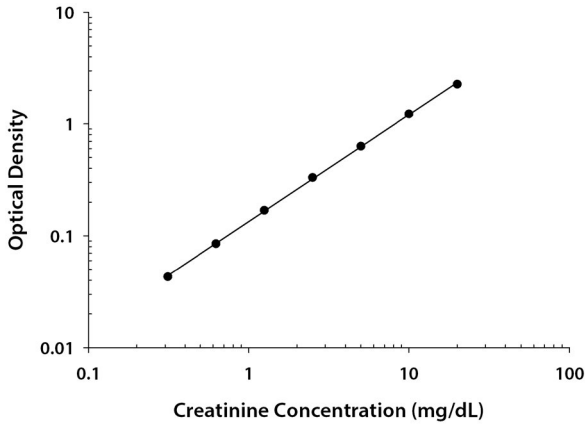
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the Creatinine concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

Since samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(mg/dL)	O.D.	Average	Corrected
0	0.085 0.088	0.087	—
0.31	0.128 0.131	0.130	0.043
0.62	0.169 0.173	0.171	0.084
1.25	0.253 0.258	0.256	0.169
2.5	0.416 0.420	0.418	0.331
5	0.715 0.722	0.719	0.632
10	1.309 1.326	1.318	1.231
20	2.356 2.362	2.359	2.272

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (mg/dL)	1.67	5.28	9.75	1.55	4.92	9.25
Standard deviation	0.053	0.168	0.339	0.085	0.259	0.366
CV (%)	3.2	3.2	3.5	5.5	5.3	4.0

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	96	92-97%

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of Creatinine were serially diluted with deionized or distilled water to produce samples with values within the dynamic range of the assay.

		Urine (n=9)
1:2	Average % of Expected	101
	Range (%)	97-105
1:4	Average % of Expected	103
	Range (%)	97-109
1:8	Average % of Expected	102
	Range (%)	96-108
1:16	Average % of Expected	101
	Range (%)	93-109

SENSITIVITY

Seventy assays were evaluated and the minimum detectable dose (MDD) of Creatinine ranged from 0.01-0.07 mg/dL. The mean MDD was 0.02 mg/dL.

The MDD was determined by subtracting two standard deviations from the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This assay is calibrated to the NIST Standard Reference Material 914a Creatinine. The conversion to SI units: mg/dL x 0.088 = mmol/L.

SAMPLE VALUES

Urine - Fifty-four samples were evaluated in this assay and were found to have Creatinine levels ranging from 13-212 mg/dL. The mean was 102 mg/dL.

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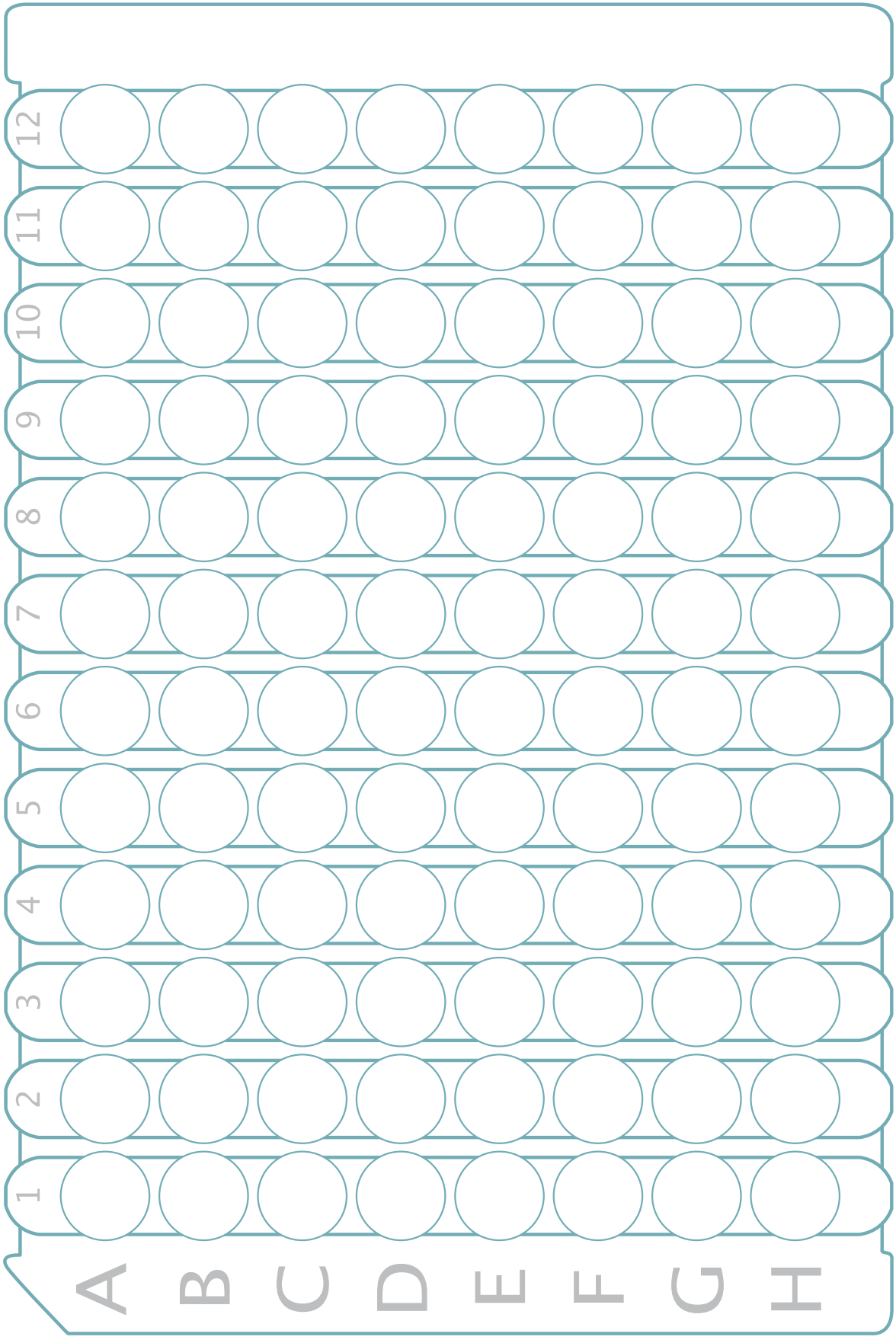
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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