ELISA PRODUCT INFORMATION & MANUAL

Creatinine Assay Kit (Colorimetric)

NBP3-07134

Sample insert for reference use only

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.

Not for diagnostic or therapeutic procedures.

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

Novus kits are guaranteed for 6 months from date of receipt

TABLE OF CONTENTS

GENERAL INFORMATION

Materials Supplied

	Reagent	Quantity	Description
	Clear Microtiter Plates	2 plates	Bag containing 2 x 96 well plates.
	Creatinine Standard	1 mL	A 100 mg/dL creatinine solution in deionized water.
	Creatinine Reagent	20 mL	-
	Plate Sealers	2 each	-

If any of the items listed above are damaged or missing, please contact our Technical Support team. We cannot accept any returns without prior authorization.

WARNING: Not for human or animal disease diagnosis or therapeutic drug use.

Precautions

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete booklet should be read and understood before attempting to use the product.

The Creatinine Reagent contains hazardous chemicals. It contains a solution of basic picric acid in a stabilizing solution. The solution should not come in contact with skin or eyes. Picric acid is an irritant and, if dried, potentially explosive. Avoid contact with metals and use large volumes of water during disposal. Take appropriate precautions when handling these reagents.

Storage

All components of this kit should be stored at 4°C until the expiration date of the kit.

Materials Needed But Not Supplied

- Distilled or deionized water.
- Colorimetric 96 well microplate reader capable of reading optical density at 490 nm, preferably with correction between 570 and 590 nm.
- Software for converting raw relative optical density readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

Please read this booklet completely prior to using the product.

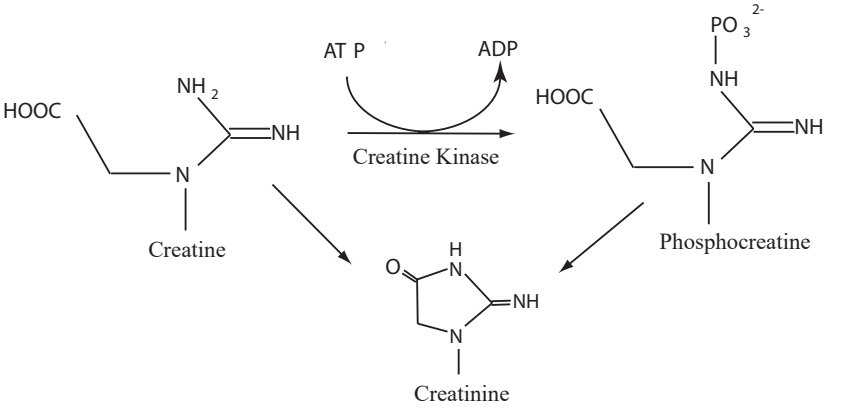
FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

INTRODUCTION

Background

Creatinine (2-amino-1-methyl-5H-imadazol-4-one) is a metabolite of phosphocreatine (p-creatine), a molecule used as a store for high-energy phosphate that can be utilized by tissues for the production of ATP[1]. Creatine either comes from the diet or synthesized from the amino acids arginine, glycine, and methionine. This occurs in the kidneys and liver, although other organ systems may be involved and species-specific differences may exist[2]. Creatine and p-creatine are converted non-enzymatically to the metabolite creatinine, 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]. Creatinine forms spontaneously from p-creatine[3]. Under normal conditions, its formation occurs at a rate that is relatively constant and as intra-individual variation is <15% from day to day, creatinine is a useful tool for normalizing the levels of other molecules found in urine. Additionally altered creatinine levels may be associated with other conditions that result in decreased renal blood flow such as diabetes and cardiovascular disease[4-6].



Assay Overview

The Creatinine Assay Kit (Colorimetric) is designed to quantitatively measure creatinine present in urine samples. Please read the complete kit booklet before performing this assay. A creatinine standard, calibrated to a NIST creatinine standard, is provided to generate a standard curve for the assay and all samples should be read off the standard curve. Standards or diluted samples are pipetted into a clear microtiter plate. The color generating reaction is initiated with the Creatinine Reagent, which is pipetted into each well. After a short incubation the intensity of the generated color is detected in a microtiter plate reader capable of measuring 490nm wavelength. The concentration of the creatine in the sample is calculated, after making a suitable correction for the dilution of the sample, using software available with most plate readers. The Jaffe reaction used in this kit has been modified to read creatinine levels in urine[7,8].

PRE-ASSAY PREPARATION

Sample Types

Sample Types Validated:
Human, Monkey, Canine, and Rat Urine

This assay has been validated for human, rat, canine and monkey urine samples. Urine samples containing visible protein or particulates should be centrifuged or filtered prior to using. Mouse urine samples are not compatible with the use of this assay to determine GFR as over half of murine urinary creatinine is from renal secretion rather than filtration9.

Sample Preparation

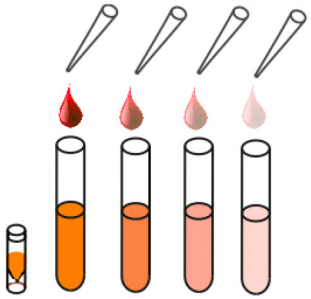
Rhesus monkey urine samples contain very low levels of creatinine and should be diluted 1:2 in water by taking one part of urine and adding to one part of water prior to using in the assay. All other urine samples must be diluted 1:20 with deionized or distilled water by taking one part of urine and adding to nineteen parts of water to obtain accurate results. Any urine samples with creatinine concentrations outside the standard curve range should be diluted further with water to obtain readings within the standard curve.
Use all diluted samples within 2 hours of preparation.

Reagent Preparation

Allow the kit reagents to come to room temperature for 30 minutes. We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine creatinine concentrations. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Standard Preparation

Label seven glass test tubes #1 through #7. Pipet 800 µL of water into tube #1 and 500 µL into tubes #2-#7. Carefully add 200 µL of the Creatinine Standard stock solution to tube #1 and vortex completely. Take 500 µL of the creatinine solution in tube #1 and add it to tube #2 and vortex completely. Add 500 µL of tube #2 to tube #3 and vortex completely. Repeat these serial dilutions for tubes #4 through #7. The concentration of creatinine in tubes 1 through 7 will be 20, 10, 5, 2.5, 1.25, 0.625 and 0.3125 mg/dL. Water will be used as a sample blank. Use all Standards within 2 hours of preparation.



	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7
Water Volume (µL)	800	500	500	500	500	500	500
Addition	Stock	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6
Volume of Addition (µL)	200	500	500	500	500	500	500
Final Concentration (mg/dL)	20	10	5	2.5	1.25	0.625	0.3125

Assay Protocol

1. Use the plate layout sheet on page 17 to aid in proper sample and standard identification.
2. Pipet 50 µL of samples, water as the blank, or standards into wells in the clear plate.
3. Add 100 µL of the Creatinine Reagent to each well using a repeater pipet.
4. Gently tap the sides of the plate to ensure adequate mixing of the reagents. Cover the plate with the plate sealer and press to seal adequately.
5. Incubate at room temperature for 30 minutes.
6. Read the optical density generated from each well in a plate reader capable of reading at 490nm.
7. Use the plate reader's built-in 4PLC software capabilities to calculate creatinine concentrations for each sample.

ANALYSIS

Calculation of Results

Average the duplicate OD readings for each standard and sample. Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic curve (4PLC) fit, after subtracting the mean OD's for the blank. The sample concentrations obtained should be multiplied by the dilution factor to obtain neat sample values.

Typical Data

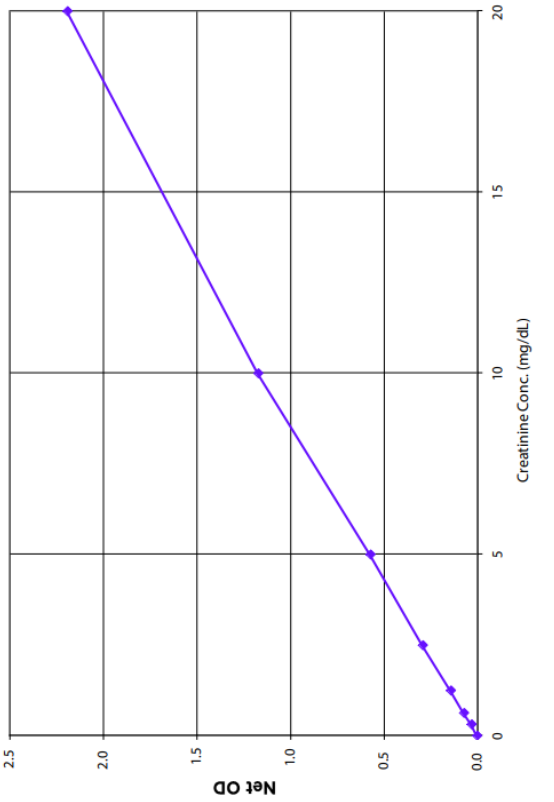
Sample	Mean OD	Net OD	Creatinine Concentration (mg/dL)
Zero	0.129	--	0
Standard 1	2.315	2.186	20
Standard 2	1.296	1.167	10
Standard 3	0.703	0.574	5
Standard 4	0.423	0.294	2.5
Standard 5	0.270	0.142	1.25
Standard 6	0.200	0.071	0.625
Standard 7	0.163	0.034	0.3125
Sample 1	0.565	0.436	3.75
Sample 2	0.178	0.049	0.43

Always run your own standard curve for calculation of results.

Do not use this data.

Conversion Factor: 1 mg/dL Creatinine is equivalent to 88.40 µM Creatinine.

Typical Standard Curves



Validation Data

Sensitivity and Limit of Detection

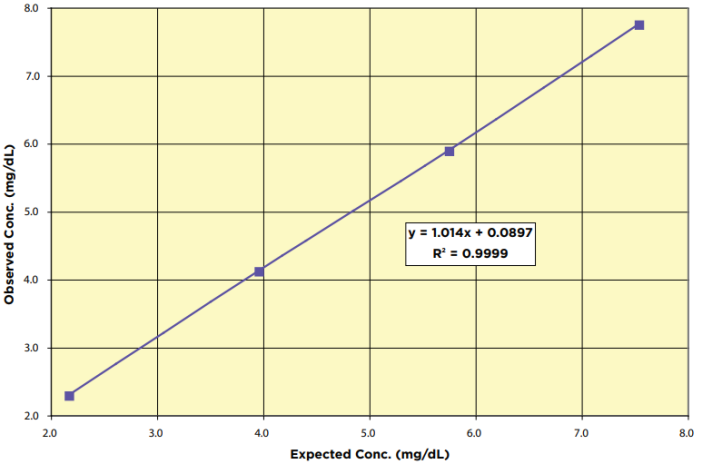
Sensitivity was calculated based on 20 replicates of water and 20 replicates of a urine sample diluted to ~ 0.040 mg/dL Creatinine concentration.
Sensitivity was determined as 0.032 mg/dL

The Limit of Detection was based on 20 replicates of water and 20 replicates of the lowest standard concentration (standard #7 0.3125mg/dL).
Limit of Detection was determined as 0.040 mg/dL

Linearity

Linearity was determined by taking two diluted 1:20 human urine samples, one with a low diluted creatinine level of 0.38 mg/dL and one with a higher diluted level of 9.33 mg/dL and mixing them in the ratios given below. The measured concentrations were compared to the expected values.

High Urine	Low Urine	Observed Concentration (mg/dL)	Expected Concentration (mg/dL)	% Recovery
100%	0%	--	9.33	--
80%	20%	7.75	7.54	102.8
60%	40%	5.89	5.75	102.4
40%	60%	4.12	3.96	104.0
20%	80%	2.29	2.17	105.5
0%	100%	--	0.38	--
			Mean Recovery	103.7%


8.0
7.0
6.0
5.0
4.0
3.0
2.0
Observed Conc. (mg/dL)
y = 1.014x + 0.0897
R² = 0.9999
2.0
3.0
4.0
5.0
6.0
7.0
8.0
Expected Conc. (mg/dL)

Intra Assay Precision

Four human urine samples were diluted 1:20 with deionized water and run in replicates of 20 in an assay. The mean and precision of the calculated creatinine concentrations were:

Sample	Cortisol Concentration (pg/mL)	%CV
1	1,174.3	6.0
2	475.9	5.6
3	177.4	14.7

Inter Assay Precision

Four human urine samples were diluted 1:20 with deionized water and run in duplicates in 20 assays run over five days by three operators. The mean and precision of the calculated creatinine concentrations were:

Sample	Cortisol Concentration (pg/mL)	%CV
1	1,188.1	7.2
2	508.7	6.3
3	199.7	10.9

Sample Values

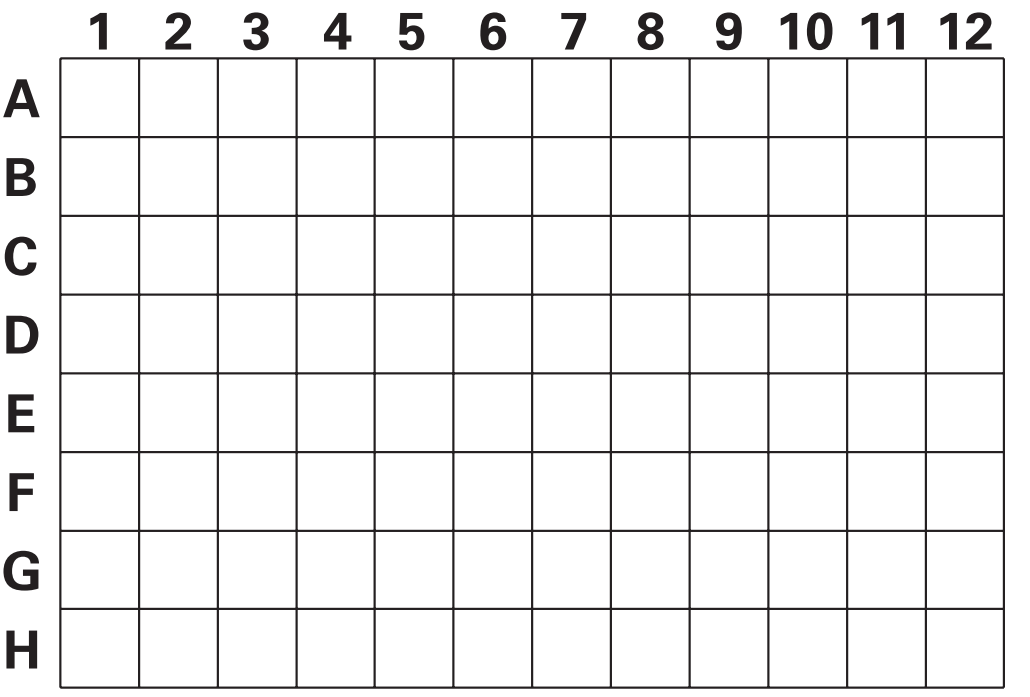
47 random clean catch human urine samples were tested in the assay. Neat urine values ranged from 17.2 to 168.9 mg/dL with an average of 90.7 mg/dL. One sample each of beagle and rat urines diluted 1:20 with water and read in the kit gave creatinine values in neat urine of 92.8 and 25.2 mg/dL respectively. A single Rhesus monkey urine, diluted either 1:2 or 1:5, averaged 2.65 mg/dL in neat urine.

Cross Reactivity

It is well known that some typical components of human urine may interfere with the Jaffe reaction for creatinine measurement in urine10,11. A diluted urine sample was spiked with 2,000 mg/dL of glucose (equivalent to 40,000 mg/dL undiluted) and tested in the kit. The unspiked diluted sample read at 8.44 mg/dL. No significant change to the measured creatinine level was seen at any glucose concentration.

References

1. Wallimann, T. et al., Biochem. J., 2000, 281, 21-40.
2. Wyss, M. and Kaddurah-Daouk, R., Physiol. Rev., 2000, 80, 1107-1213.
3. Raja Iyengar, M. et al., J. Biol. Chem, 1985, 260, 7562-7567.
4. Manjunath, G. et al., Postgrad. Med. 2001, 110, 55-62.
5. Gross, J.L. et al., Diabetes Care, 2005, 28, 164-176.
6. Anavekar, N.S. et al., New Engl. J. Med., 2004, 351, 1285-1295.
7. Slot, C, Scand. J. Clin. Lab. Invest., 1965, 17, 381-383.
8. Heinegard, D. and Tederstrom, G., Clin. Chem. Acta, 1973, 43, 305-310.
9. Eisner C., et al., Kidney International, Major contribution of tubular secretion to creatinine clearance in mice. 2010, 77(6):519-26.
10. Cook, J.G., Ann Clin. Biochem., 1975, 12, 219-232.
11. Young, D.D., in "Effects of Drugs on Clinical laboratory Tests", 1990.


1 2 3 4 5 6 7 8 9 10 11 12
A
B
C
D
E
F
G
H

Warranty and Limitation of Remedy

Novus Biologicals makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Novus Biologicals **warrants only** to the original customer that the material will meet our specifications at the time of delivery. Novus Biologicals will carry out its delivery obligations with due care and skill. Thus, in no event will Novus Biologicals have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Novus Biologicals is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Novus Biologicals, its directors or its employees.

Buyer's **exclusive remedy** and Novus Biologicals' sole liability hereunder shall be limited to a refund of the purchase price, or at Novus Biologicals' option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Novus Biologicals within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Refund Policy located on our website and in our catalog.

Contact Information

Phone: 303-730-1950

Fax: 303-730-1966

E-Mail: technical@novusbio.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).