ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES
N-CAL™ KIT

NCal™ International Standard Kit

DetectX®

Urinary Creatinine
Detection Kit

2 Plate Kit Catalog Number K002-H1
10 Plate Kit Catalog Number K002-H5

Species Independent

Sample Types Validated:

Human, Monkey, Dog, and Rat Urine

Calibrated to NIST Standard Reference Material Lot No. 914a

Please read this insert completely prior to using the product.
For research use only. Not for use in diagnostic procedures.

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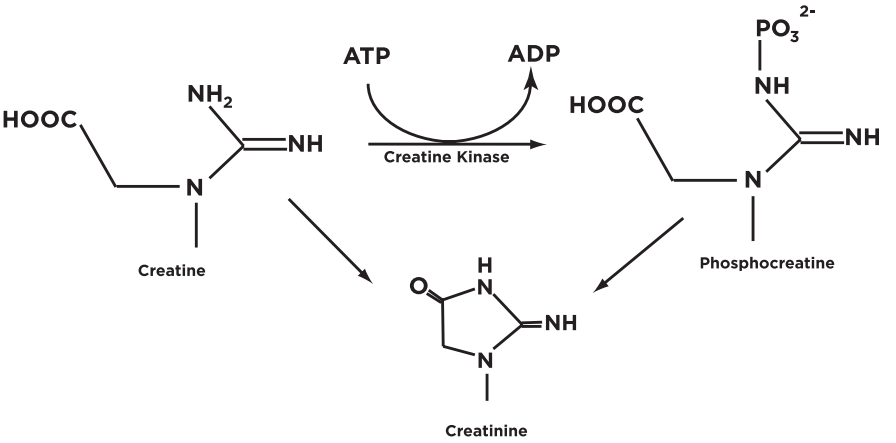
BACKGROUND

Creatinine (2-amino-1-methyl-5H-imidazol-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].



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.

ASSAY PRINCIPLE

The DetectX® Urinary Creatinine Kit is designed to quantitatively measure creatinine present in urine samples. Please read the complete kit insert 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 DetectX® 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].

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.

RELATED PRODUCTS

Kits	Catalog No.
Cortisol ELISA Kit	K003-H1/H5
Formaldehyde Fluorescent Detection Kit	K001-F1
Glutathione Colorimetric Detection Kit	K006-H1
Glutathione Fluorescent Detection Kits	K006-F1/F5
Retinol Binding Protein ELISA Kits	K062-H1/H5
Urea Nitrogen (BUN) Detection Kits	K024-H1/H5

SUPPLIED COMPONENTS

Clear 96 Well Plates

Bag containing 2 by 96 well plates or 2 bags each containing 5 by 96 well plates

2 plates	(K002-H1 Kit)	Catalog Number X003-2EA
2 by 5 plates	(K002-H5 Kit)	Catalog Number X003-5EA

Creatinine Standard

A 100 mg/dL creatinine solution in deionized water.

1 mL	(K002-H1 Kit)	Catalog Number C003-1ML
2 by 1 mL	(K002-H5 Kit)	Catalog Number C003-1ML

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DetectX® Creatinine Reagent

20 mL	(K002-H1 Kit)	Catalog Number C004-20ML
2 by 50 mL	(K002-H5 Kit)	Catalog Number C004-50ML

Plate Sealers

2 each	(K002-H1 Kit)	Catalog Number X002-1EA
10 each	(K002-H5 Kit)	Catalog Number X002-1EA

STORAGE INSTRUCTIONS

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

OTHER MATERIALS REQUIRED

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.

PRECAUTIONS

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert 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.

SAMPLE TYPES

This assay has been validated for human, rat, dog 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 filtration[9].

For measuring Creatinine in serum or plasma samples please refer to the DetectX® Serum Creatinine Detection kit, Catalog Number KB02-H1.

9. Eisner C., et al., Kidney International, Major contribution of tubular secretion to creatinine clearance in mice. 2010, 77(6):519-26.

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.

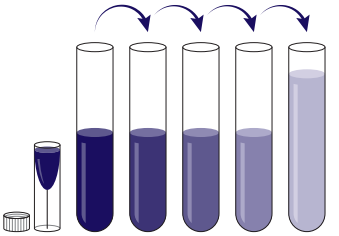
Use all diluted samples within 2 hours of preparation.

REAGENT PREPARATION

Allow the kit reagents to come to room temperature for 30 minutes. 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.

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

ASSAY PROTOCOL

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine creatinine concentrations.

1. Use the plate layout sheet on the back page 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 DetectX® 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 490 nm.
7. Use the plate reader's built-in 4PLC software capabilities to calculate creatinine concentrations for each sample.

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.

Or use the online tool from MyAssays to calculate the data:
www.myassays.com/arbor-assays-creatinine-urinary-detection-kit.assay

TYPICAL DATA

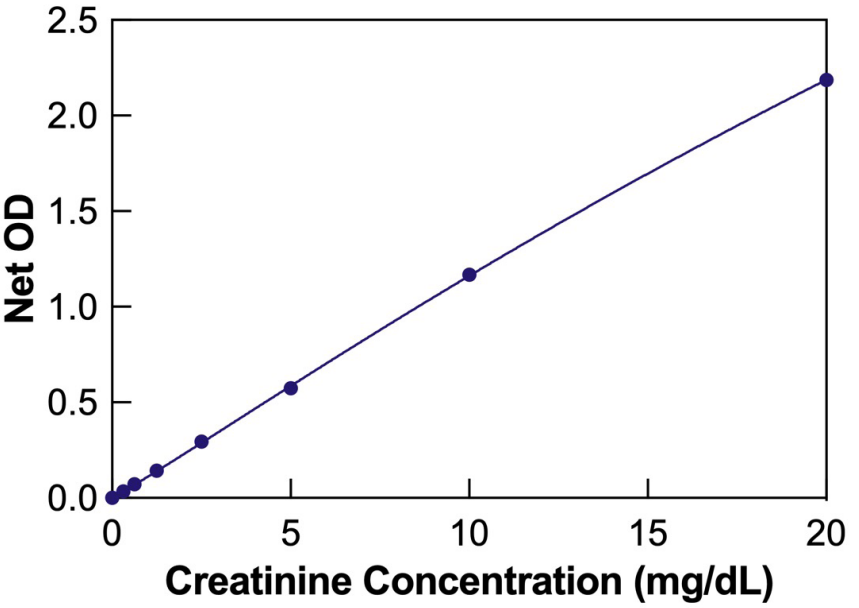
Sample	Mean OD	Net OD	Creatinine Conc. (mg/dL)
Zero	0.129	--	0
Standard 1	2.315	2.186	20
Standard 2	1.296	1.167	10
Standard 3	0.703	0.574	5
Standard 4	0.423	0.294	2.5
Standard 5	0.270	0.142	1.25
Standard 6	0.200	0.071	0.625
Standard 7	0.163	0.034	0.3125
Sample 1	0.565	0.436	3.75
Sample 2	0.178	0.049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

Always run your own standard curve for calculation of results. Do not use this data.

Creatinine standard calibrated to NIST Standard Reference Material Lot Number 914a

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

Typical Standard Curve



Always run your own standard curves for calculation of results. Do not use this data.

VALIDATION DATA

Sensitivity and Limit of Detection

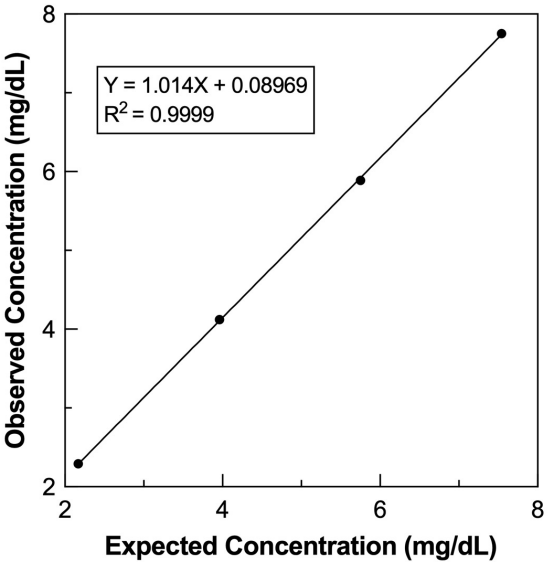
Sensitivity was calculated by comparing the OD's for twenty wells run for each of the zero and standard #7. The detection limit was determined at two (2) standard deviations from the zero along the standard curve. **Sensitivity was determined as 0.019 mg/dL.**

The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for twenty wells run for each of the zero standard and a low concentration human urine sample. **Limit of Detection was determined as 0.037 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 Conc. (mg/dL)	Expected Conc. (mg/dL)	% Recovery
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
			Mean Recovery	**103.7%**



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	Creatinine Conc. (mg/dL)	%CV
1	8.92	2.8
2	4.08	1.3
3	1.94	2.5
4	1.11	3.0

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	Creatinine Conc. (mg/dL)	%CV
1	9.04	2.3
2	4.18	2.7
3	2.03	3.9
4	1.18	3.7

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 AND INTERFERENTS

It is well known that some typical components of human urine may interfere with the Jaffe reaction for creatinine measurement in urine[10,11]. 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.

10. Cook, J.G., Ann Clin. Biochem., 1975, 12, 219-232.
11. Young, D.D., in “Effects of Drugs on Clinical laboratory Tests”, 1990.

LIMITED WARRANTY

Arbor Assays warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

CONTACT INFORMATION

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OFFICIAL SUPPLIER TO ISWE

Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with assay kits and reagents for wildlife conservation research.

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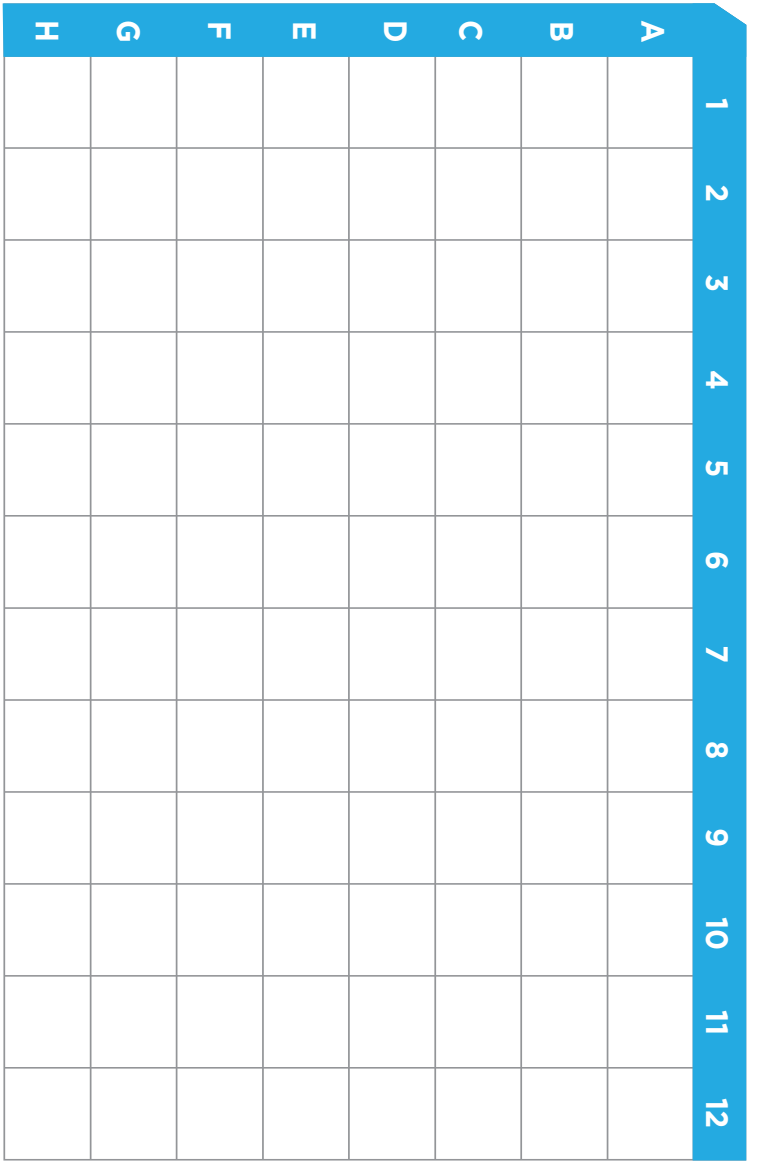

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