ARBOR ASSAYS™

DetectX® Serum Creatinine

Detection Kit

2 Plate Kit – Catalog No. KB02-H1
4 Plate Kit – Catalog No. KB02-H2

Species Independent

Sample Types Tested:
Serum and Plasma

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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SUPPLIED COMPONENTS & STORAGE

		KB02-H1	KB02-H2	Description
Clear 96 Well Half Area Plates	Quantity	2	4	Corning CoStar Plate 3695
	Catalog No.	X018-2EA	X018-4EA	
Creatinine Standard	Volume	100 µL	1 mL	100 mg/dL Creatinine solution in water. Calibrated to NIST Standard Reference Material Lot Number 914a.
	Catalog No.	C003-100UL	C003-1ML	
Assay Diluent	Volume	6 mL	12 mL	Serum-specific diluent buffer
	Catalog No.	X017-6ML	X017-12ML	
DetectX® Creatinine Reagent	Volume	20 mL	50 mL	Contains picric acid CAUSTIC
	Catalog No.	C004-20ML	C004-50ML	

This this kit should be stored at 4°C up to the expiration date on the kit label.

OTHER MATERIALS REQUIRED

- Distilled or deionized water
- Adjustable pipettes with disposable tips. Repeater pipettes or multichannel pipettes with corresponding tips are also recommended.
- Glass or high-quality polypropylene test tubes for standard and sample preparation
- A plate reader capable of measuring absorbance at 490 nm
- Software for converting optical density (OD) readings from the plate reader and carrying out a linear regression. Contact your plate reader manufacturer for details.
- Timer set to countdown from 30 minutes

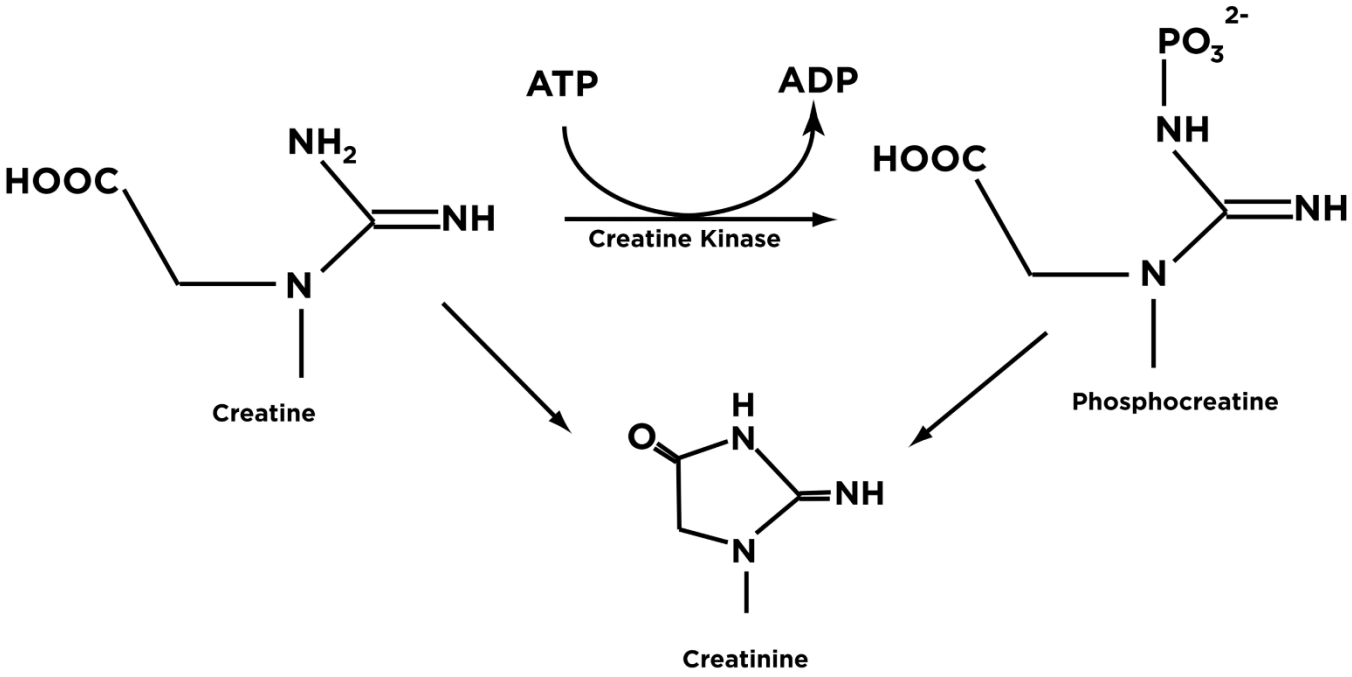
PRECAUTIONS

- Read this insert completely prior to using the product.
- This kit may not perform as described if any reagent or procedure is replaced or modified. Do not interchange reagents from different kit lots.
- Take appropriate safety precautions, such as: avoid breathing fumes, wear personal protective equipment (gloves, clothing, eye and face protection), and familiarize yourself with SDS documents.
    - https://www.arborassays.com/documentation/msds/KB02-H_MSDS.pdf
- The **Creatinine Reagent contains a solution of basic picric acid** in 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.

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 to produce 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 is 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 PRINCIPLE

The DetectX® Serum Creatinine Kit is designed to quantitatively measure Creatinine present in serum and EDTA/heparin plasma 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 samples are pipetted into a clear microtiter plate. An assay diluent is added to all standards, controls, and samples. The color generating reaction is initiated with the DetectX® Creatinine Reagent, which is pipetted into each well.

The assay utilizes a kinetic absorbance method to overcome interference by colored compounds in serum. The absorbance of the colored product is read after 1 minute in a microtiter plate reader capable of measuring 490nm wavelength. At 30 minutes the optical density is read again. The concentration of Creatinine is calculated using the difference in optical density between these two time points and a standard curve. The Jaffe reaction used in this kit has been modified to read Creatinine levels in serum[7].

For measuring Creatinine in urine samples, please refer to our DetectX® Urinary Creatinine Detection kits, Catalog Number K002-H.

REAGENT PREPARATION

Allow all kit reagents to come to room temperature for 30 minutes. Room temperature for this assay is defined as 22°C – 24°C.

SAMPLE PREPARATION

Upon collection, all samples should be frozen rapidly and stored at -80°C until testing.

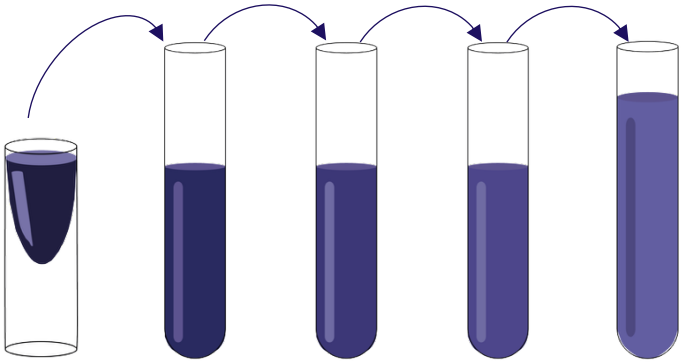
Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Hemolyzed or lipemic samples should not be used with this kit.

Sample Type	Procedure
Serum and EDTA/Heparin Plasma	• Centrifuge sample for 15 minutes at 14,000 rpm

STANDARD PREPARATION

1. Label tubes Standard 1 through Standard 4.
2. Add 240 µL water to Standard 1 tube.
3. Add 100 µL water to Standards 2 – 4 tubes.
4. Briefly vortex the Creatinine Standard stock and add 10 µL to Standard 1 tube. Vortex thoroughly.
5. Transfer 100 µL of the mixed solution from Standard 1 into Standard 2 tube. Vortex thoroughly.
6. Continue serially diluting into the remaining tubes. This process and the final concentrations are further summarized in the table below.
7. Use water as a Blank of 0 mg/dL.



	Standard 1	Standard 2	Standard 3	Standard 4
Water Volume (µL)	240	100	100	100
Addition	Stock	Std 1	Std 2	Std 3
Volume of Addition (µL)	10	100	100	100
Final Concentration (mg/dL)	4.0	2.0	1.0	0.5

⚠ **Use all Standards within 2 hours of dilution.**

ASSAY PROTOCOL

Before You Begin:

- **Room Temperature for this assay is defined as 22ºC – 24ºC.**
- **Ensure all reagents have been warmed to room temperature.**
- **Run all standards and samples in duplicate.**
- Use the blank plate template on the back page of this booklet to design your plate layout and aid in proper sample and standard identification.
- Set plate reader to read absorbance at 490 nm for a 96-well Corning Costar 3695 plate. See ArborAssays.com for plate dimension data.
- Set a timer to count down 30 minutes.
    - The plate will be read twice, at both 1 and 30 minutes.

1. Add 25 µL of Samples, Standards, or water (Blank) into duplicate wells.
2. Add 25 µL of Assay Diluent to all wells.
3. Observe wells, checking for bubbles. If bubbles are present, tap the plate gently to until no bubbles remain.
4. Add 100 µL of the DetectX® Creatinine Reagent to each well. Start the 30-minute timer immediately after adding Creatinine Reagent to the final well. The addition of Creatinine Reagent initiates the reaction. Incubate the reaction at room temperature.
5. At 1 minute, read the optical density generated from each well at 490 nm (T-1min).
6. At 30 minutes, read the optical density generated from each well at 490 nm again (T-30min).

CALCULATION OF RESULTS

Follow the instructions below or use the online tool: https://myassays.com/assay.aspx?id=914

1. Use linear regression software to calculate the Creatinine concentration for each sample. Gather all raw data OD readings for both T-1min and T-30min for each Sample, Standard, and Blank.
2. Average the duplicate OD readings for each Sample and Standard (Mean OD) for both the T-1min and T-30min readings.

EXAMPLE:

Sample	Replicate 1 OD	Replicate 2 OD	Mean OD
Standard 1 (T-1min)	0.400	0.406	0.403
Standard 1 (T-30min)	0.782	0.790	0.786
Sample 1 (T-1min)	0.649	0.669	0.659
Sample 1 (T-30min)	0.980	0.982	0.981

3. Subtract the Mean OD at T-1min from the Mean OD at T-30min for all Samples and Standards (∆OD)

EXAMPLE:

Sample	Mean OD T-1min	Mean OD T-30min	∆OD
Standard 1	0.403	0.786	0.383
Sample 1	0.659	0.981	0.322

4. Plot the standard curve with ∆OD for the Standards on the y-axis and Creatinine concentration (mg/dL) on the x-axis. Perform a linear regression.

   Use the slope and Y-intercept of the regression line, together with the ∆OD to calculate the Creatinine concentrations in diluted samples using the equation below. If diluted Creatinine concentrations are outside of the range of the Standards, the Sample should be prepared again at a more appropriate dilution.

$$\text{Sample Creatinine Concentration (mg/dL)} = \frac{(\Delta OD) - (\text{Y-intercept})}{\text{Slope}}$$

EXAMPLE:

Sample	∆OD	Sample Creatinine Concentration (mg/dL)
Sample 1	0.322	3.37

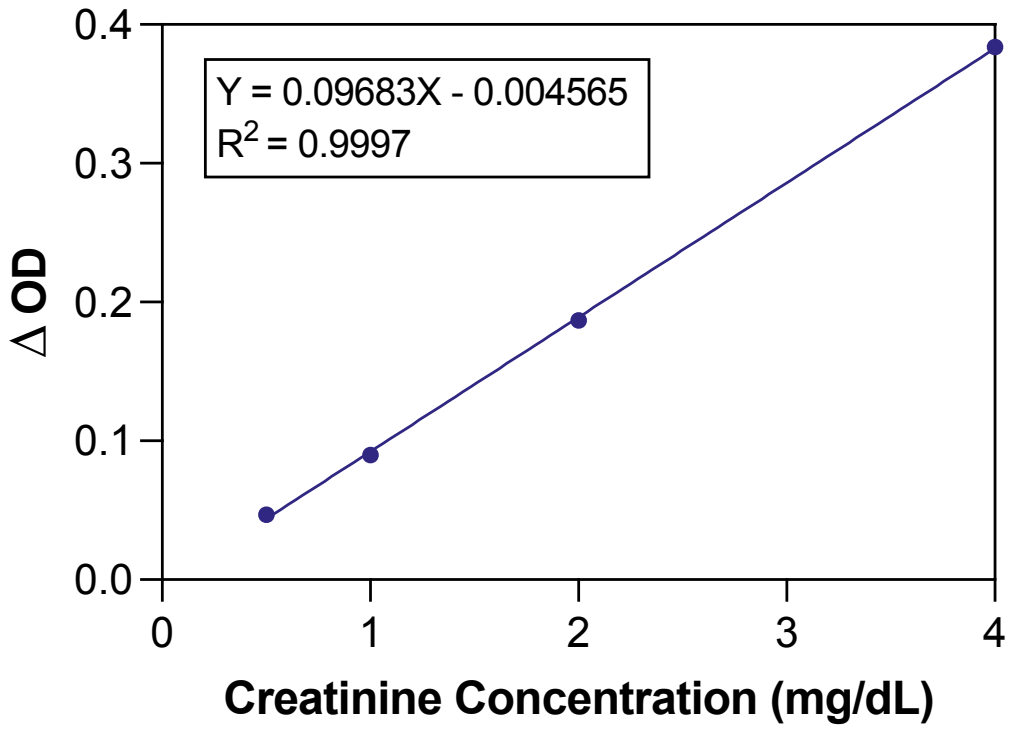
TYPICAL DATA

⚠ **Always run your own standard curve. This data should NOT be used to interpret results.**

Sample	ΔOD	Creatinine Concentration (mg/dL)
Standard 1	0.384	4.0
Standard 2	0.187	2.0
Standard 3	0.090	1.0
Standard 4	0.047	0.50
Sample 1	0.322	3.37
Sample 2	0.126	1.36

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

Typical Standard Curve



VALIDATION DATA

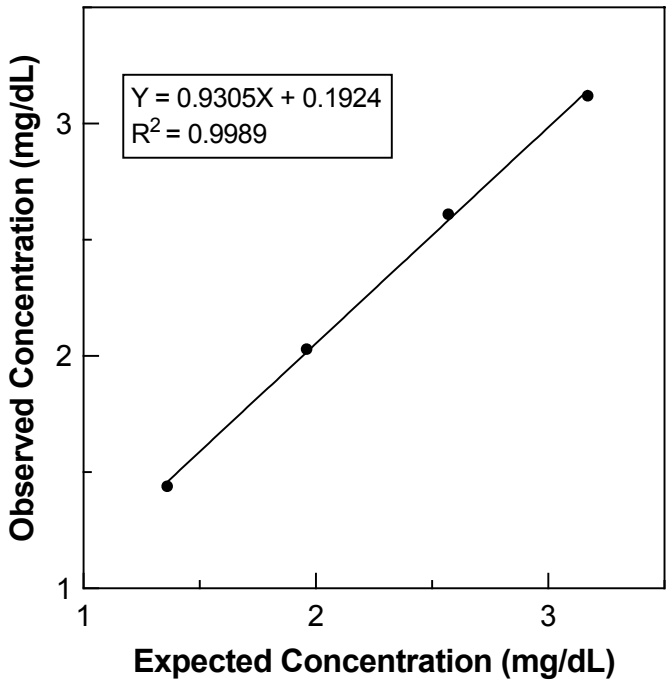
Sensitivity

Sensitivity was calculated by comparing the Delta ODs obtained for twenty wells run for each of the zero and standard #4. The detection limit was determined at two (2) standard deviations from the zero along the standard curve.
Sensitivity was determined as 0.081 mg/dL.

Linearity

Linearity was determined by taking two human serum samples, one with a low diluted Creatinine level of 0.75 mg/dL and one with a higher level of 3.78 mg/dL and mixing them in ratios given below. The measured concentrations were compared to the expected values.

Low Serum	High Serum	Expected Concentration (mg/dL)	Observed Concentration (mg/dL)	% Recovery
80%	20%	1.36	1.44	106.2
60%	40%	1.96	2.03	103.5
40%	60%	2.57	2.61	101.6
20%	80%	3.17	3.12	98.3
			Mean Recovery	**102.4%**



Intra Assay and Inter Assay Precision

For intra assay precision, three human serum samples were run in replicates of 20 in one assay. For inter assay precision, duplicates of three human serum samples were run in nineteen assays over two years by four operators. The %CV represents the variation in concentration (not optical density) as determined using a standard curve.

	Intra Assay Precision		Inter Assay Precision	
Sample	Creatinine Concentration (mg/dL)	% CV	Creatinine Concentration (mg/dL)	% CV
1	0.99	7.9	0.91	9.6
2	1.50	6.3	1.26	7.3
3	3.82	4.5	3.51	8.0

SAMPLE VALUES

Eleven serum samples from a variety of different species were tested in the assay.

Sample Type	Average Concentration (mg/dL)	Concentration Range (mg/dL)
Serum (n=11)	1.00	0.78 - 1.45

INTERFERENCE

It is well known that some typical components of serum may interfere with the Jaffe reaction for Creatinine measurement[7,8]. A serum sample was spiked with varying concentrations of bilirubin and tested in the assay. Bilirubin level in normal serum is between 0.2 and 1.0 mg/dL[9]. The unspiked sample read at 0.86 mg/dL. No significant change to the measured Creatinine level was seen up to an additional 1.0 mg/dL of bilirubin.

Hemolyzed or lipemic samples should not be used with this kit. Hemolyzed samples have shown a decrease in Creatinine concentration with increasing hemoglobin, whereas lipemic samples have been shown to yield artificially high Creatinine concentrations.

TROUBLESHOOTING

Issue	Possible Cause & Solution
Reagent Shortage	• Check under the cap for additional reagent. Pulse spin reagent containers to collect contents prior to opening when possible. • When using a multichannel pipette, return unused reagent to container for later use.
Erratic Values	• Prerinse pipet tips with desired reagent prior to aspirating the required volume. • Deliver volume with care to prevent splashing into adjacent wells.
High Background	• Reagent contamination during assay setup.
Low Signal	• Verify the plate reader wavelength is 490 nm.
Unused Wells	• If you do not use the whole plate, mark the wells that have been used. The unused wells can be used at a later date. **Do not wash and reuse wells**.

CITATIONS

1. Wallimann, T., Wyss, M., Brdiczka, D., Nicolay, K., & Eppenberger, H. M. (1992). Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: the 'phosphocreatine circuit' for cellular energy homeostasis. Biochemical Journal, 281(Pt 1), 21.
2. Wyss, M., & Kaddurah-Daouk, R. (2000). Creatine and Creatinine metabolism. Physiological reviews, 80(3), 1107-1213.
3. Iyengar, M. R., Coleman, D. W., & Butler, T. M. (1985). PhosphoCreatinine, a high-energy phosphate in muscle, spontaneously forms phosphocreatine and Creatinine under physiological conditions. Journal of Biological Chemistry, 260(12), 7562-7567.
4. Manjunath, G., Sarnak, M. J., & Levey, A. S. (2001). Estimating the glomerular filtration rate: dos and don'ts for assessing kidney function. *Postgraduate medicine*, *110*(6), 55-62.
5. Gross, J. L., De Azevedo, M. J., Silveiro, S. P., Canani, L. H., Caramori, M. L., & Zelmanovitz, T. (2005). Diabetic nephropathy: diagnosis, prevention, and treatment. *Diabetes care*, *28*(1), 164-176.
6. Anavekar, N. S., McMurray, J. J., Velazquez, E. J., Solomon, S. D., Kober, L., Rouleau, J. L., ... & Pfeffer, M. A. (2004). Relation between renal dysfunction and cardiovascular outcomes after myocardial infarction. New England Journal of Medicine, 351(13), 1285-1295.
7. Young, D.S. (1990) Effects of Drugs on Clinical Laboratory Tests. 3rd Edition, AACC Press, Washington DC, 6-12.
8. Cook, J. G. H. (1975). Factors Influencing the Assay of Creatinine: Prepared for the Association of Clinical Biochemists' Scientific and Technical Committee. Annals of clinical biochemistry, 12(1-6), 219-232.
9. Tietz, N.W. (1986) Textbook of Clinical Chemistry, W.B.Saunders Company, Philadelphia.

RELATED PRODUCTS

Kits	Catalog No.
Human Cystatin C ELISA Kit	K012-H1
Urinary Creatinine Detection Kits	K002-H1/H5
Hemoglobin High Sensitivity Detection Kits	K013-HX1/HX5
Retinol Binding Protein (RBP) Multi-Format ELISA Kits	K062-H1/H5

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.

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CONTACT INFORMATION

For details concerning this kit or to order any of our products please contact us.

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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.

PLATE LAYOUT

H	G	F	E	D	C	B	A	
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