Serum Creatinine ELISA KIT

Cat. # 100-300-SCR (96 tests)
Cat. # 100-305-SCR (2x96 tests)

For Quantitative Determination of Creatinine in mouse, rat, or human (applicable to all species)

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

6203 Woodlake Center Drive • San Antonio• Texas 78244 • USA.

Phone (210) 561-9515 • Fax (210) 561-9544

Toll Free (800) 786-5777

Email: service@4adi.com

Web Site: www.4adi.com

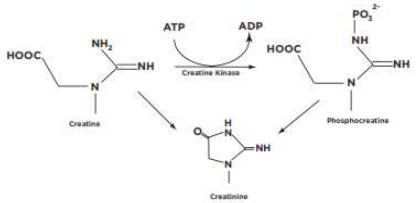
ELISA KIT # 100-300-SCR (96 tests) or 100-305-SCR (2x96 tests)
For Quantitative Determination of creatinine in serum

Kit Components	(96 tests)	(2x 96 tests)
microwell strip plate (8 wells x 12 or (96 wells), Ready-to-use	1 plate	2 plates
Creatinine Stock Standard solution (100 mg/dL) calibrated to NIST refs #914a (#100300CR-1)	100 ul	200 ul
Assay diluent #(#100300CR-2	6 ml	11 ml
Creatinine Reagent solution	20 ml	50 ml
Complete Instruction Manual	M-1500	M-1500
Store kit at 4oC and allow to warm at room for at least 30 min prior to use.		

Introduction

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 . 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. 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 . 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

ADI's creatinine ELISA kit provides for the measurement of creatinine in sera of animals or humans.



Creatinine ELISA plate template

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

Creatinine ELISA cat# _____**; # of test:**_____

Lot #/: _____ **Expiration:** _____

Assay Date: _____

Start time:_____ End time: _____

Operator: _____ _____

PERFORMANCE CHARACTERISTICS

1. DETECTION LIMIT

Based on twenty replicate determinations of the zero standard, the minimum concentration of creatinine detected using this assay is 0.081 mg/dL. The detection limit is defined as the value deviating by 2 SD from the zero standard.

2. PRECISION

Intra-assay precision:

Three serum samples (0.99, 1.5, 3.82 mg/dL) were run in 20 replicates in an assay. The samples showed good intra-assay precision (4.8-7.9 %CV).

Inter-assay precision:

Three serum samples (0.99, 1.5, 3.82 mg/dL) were run in duplicate in ten independent assays. The samples showed good inter-assay precision (7-9.6 %CV).

3. 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	Observed Conc. (mg/dL)	Expected Conc. (mg/dL)	% Recovery
80%	20%	1.44	1.6	106.2%
60%	40%	203	1.96	103.5%
40%	60%	2.61	2.57	101.6%
20%	80%	3.12	3.17	98.3%
			Mean	102.4%

4. Sample values

Eleven serum samples from a variety of different species were tested in the assay. Values ranged from 0.78 to 1.45 mg/dL with an average of 1.00 mg/dL.

5. Cross reactivity and interference

It is well known that some typical components of serum may interfere with the Jaffe reaction for creatinine measurement. 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. 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.

6. Species reactivity

Creatinine is the same in all species. The assay can be used in mouse, rat, human or other species.

General References: Wang MC (1979) Invest. Urol. 17, 159; Frankel AE (1982) Cancer Res. 42, 3714; Papsider LD (1980) Cancer Res. 40, 2428. Ponts JE (1982) J. Urol. 128, 1216; Killan CS (1985) Cancer Res. 45, 886; Kuriyam M (1980) Cancer Res. 40, 4658; Kuriyam M (1982) J Natl. Cancer 68, 99; Schiffman RB (1987) Clin Chem. 33, 2086

PRINCIPLE OF THE TEST

Serum creatinine ELISA kit is designed to quantitatively measure creatinine present in serum 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 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 delta of the optical density readings at 30 and 1 minute compared to the curve generated from the standards, or by using the Excel worksheet available for free download at our web site. The Jaffe reaction used in this kit has been modified to read creatinine levels in serum. 8

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-200 ul) and multichannel pipet with disposable plastic tips, Reagent troughs, plate washer (recommended) and ELISA plate Reader capable of reading at 490nm..

PRECAUTIONS

The Alpha Diagnostic International creatinine ELISA test is intended for *in vitro research* use only. The reagents contain thimerosal as preservative; necessary care should be taken when disposing solutions. The Control Serum has been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site. Thimerosal and Prolcin-300.

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation at room temperature. Do not heat inactivate the serum.. If sera can not be immediately assayed , these could be stored at -20^{O}C for up to six months. EDTA and heparinized plasma can also be used. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

All samples should be centrifuged for 15 minutes at 14,000 rpm in an Eppendorf type centrifuge prior to running in the assay.

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. ADI also have Hemoglobin Detection kit for measuring Hb levels.

Preparation of the reagent:

Prepare working standards fresh before the assay (see page 3).

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8^{O}C until the expiration date printed on the label. The whole kit stability is usually 12 months from the date of shipping under appropriate storage conditions. Do not expose these solutions to strong light during storage or use.

TEST PROCEDURE - *ALLOW **ALL** REAGENTS TO REACH ROOM TEMPERATURE (25-30oC) BEFORE USE. Addition of cold reagents will reduce reaction rate and less color.*

Preparation of Working standards

First Std A is prepared from the stock by dilution 1:25 with water and then it is further diluted 2-fold for other stds B-D using the following scheme.

Working Stds	Stock Std @100 mg/dL	Water (ul)	Total Volume (ul)	Creatinine Final Conc (mg/dL)
Std. A	10 ul of Stock Std.	240 ul	250 ul	4
Std B	100 ul of Std A	100 ul	200 ul	2
Std C	100 ul of Std B	100 ul	200 ul	1
Std D	100 ul of Std C	100 ul	200 ul	0.5
Blank	-	200 ul	200 ul	0
Note: Do not store working standards for more than 2 hours and prepare fresh working standards from the stock. Need 25 ul of each std in duplicate or 50 ul total per assay.				

1. Remove required # strips and arrange them on the ELISA frame. unused strips can be stored in the supplied plastic bag. The ELISA plate frame can be saved after the test to be used again if partial plate was used for the assay. It is highly recommended to use the stds/sample layout sheet.

2. Set the ELISA reader to read at 490nm. You will need to read the plate immediately after the addition of all reagents at 1 min and after 30 mins.

3. Pipet **25 ul of standards, control, and serum samples** into appropriate wells in *duplicate*.

4. Add **25 ul of assay diluent** into each well**.**

5. Dispense **100 ul Creatinine Reagent** into each well**.** Mix gently for 5-10 seconds. It is recommended to use a repeater pipette or mutliwell pipette to quick dispense reagent.

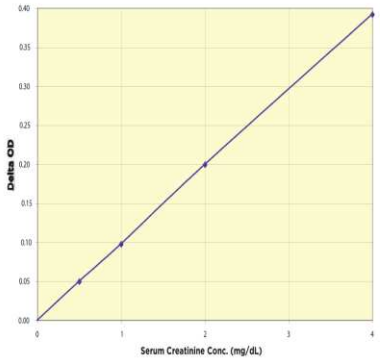
6. Incubate the plate for **30 minutes** at room temperature.

7. At 1 minute read the plate at 490nm and again at 30 min. You will need to subtract the 1 minute values from the 30 min reading to get the net delta A490.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples (mg/dL)		Net Delta A_{490nm}	Calculated Conc. (ng/ml)
A1, A2	**Std. A** (0.5)		0.051	
B1, B2	**Std. B** (1.0)		0.099	
C1, C2	**Std. C** (2)		0.201	
D1, D2	**Std. D** (4)		0.393	
G1, G2	**Sample 1**		0.135	1.36

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



A typical standard assay curve (do not use this for calculating sample values)

Calculation of Results

Subtract the average A490 of the standards/samples at 1 minute from the average A490 of the standards/samples at 30 minutes and plot the result (Average Delta OD) versus the creatinine concentration of the standards. Generate a linear regression line and use the equation, y=mx+b (y=Average delta OD; x=Creatinine Concentration: m=slope and b= intercept) to calculate the concentrations in the unknown samples.

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

Conversion Factor: 1 mg/dL Creatinine is equivalent to 88.40 **µ**M Creatinine