MA-0121

Creatinine Assay

(100 wells, Colorimetric, OD 570 nm or Fluorometric, Ex/Em = 538/587 nm, Store at -20°C)

Background Information:

Creatinine is a breakdown product of muscle and protein metabolism. Creatinine is produced and excreted at a constant rate, and as such is used as an internal control for the rate of formation or breakdown of a variety of metabolic intermediates. Since urine production rates can vary due to a number of factors, the creatinine concentration corrects for this and is used to normalize urine concentrations of other compounds. Creatinine is excreted via glomerular filtration and secretion from the proximal tubules. Blood creatinine levels increase if glomerular filtration decreases and are used to determine the estimated GFR (eGFR). AkriviBio's Creatinine Assay Kit provides a simple, sensitive measure of creatinine concentration in a variety of biological samples. In the assay, creatinine broken down with the eventual formation of hydrogen peroxide which is used by peroxidase to convert ADHP to the highly colored and fluorescent resorufin. The assay can quantify less than 1 nmole per well by absorbance and below 0.1 nmole per well by fluorescence.

Assay Principle:

1 – Creatinine is converted to creatine by creatininase
2 – Creatine is converted to sarcosine by creatinase
3 – Sarcosine is oxidized to glycine, formaldehyde and hydrogen peroxide
4 – Peroxidase utilizes hydrogen peroxide to oxidize ADHP to resorufin

Assay Components:

Assay Buffer	25 ml	WM	MA-0121-A
ADHP Solution	0.2 ml	Red	MA-0121-B
Creatinase	1 vial	Blue	MA-0121-C
Creatininase	1 vial	Violet	MA-0121-D
Sarcosine Oxidase/Peroxidase	1 vial	Green	MA-0121-E
Creatinine Standard	10 µmol	Yellow	MA-0121-F

Storage and Handling:

Store kit at -20°C. Centrifuge all vials briefly, prior to opening. Bring all components to room temperature before use. Store

- **Assay Buffer:** Ready to use as supplied. Store at 4°C.
- **ADHP Solution:** Ready to use as supplied. Store at 4°C.
- **Creatininase, Creatinase, Sarcosine Oxidase/Peroxidase:** Reconstitute each using 220 µl of Assay Buffer. Dissolve then place on ice. If you plan to use the Assay several times over a period of time, aliquot the enzyme solutions into convenient portions and freeze to prevent multiple freeze/thaw cycles which accelerates enzyme denaturation. Store at -20°C after reconstitution.
- **Creatinine Standard:** Reconstitute with 100 µl of dH_2O. Store reconstituted standard at -20°C.

Assay Protocol:

1. Standard Curve: Dilute the Creatinine Standard 100X by taking 10 µl to a tube containing 990 µl of Assay Buffer giving a 1 mM solution. For an absorbance-based assay, transfer 0 – 2 – 4 – 6 – 8 – 10 µl to a series of wells in a 96 well plate giving 0 – 2 – 4 – 6 – 8 – 10 nmoles per well. Adjust all well volumes to 50 µl with Assay Buffer. For a fluorescence-based assay, dilute the standard 10X further to 0.1 mM. Transfer 0 – 2 – 4 – 6 – 8 – 10 µl to a series of wells giving 0 – 200 – 400 – 600 – 800 – 1000 pmoles per well. Adjust all well to 50 µl.

2. Sample Preparation: Creatinine samples are typically liquid (urine, serum, CSF, etc.). Samples should be deproteinized using a 10 kDa filter. For more accurate results, add at least 5-10 and up to 50 µl of each sample to wells in a 96 well plate. Adjust the volume to 50 µl/well with Assay Buffer.

Notes:

- Serum creatinine normal range is about 62 – 115 µM in men and 53 – 97 µM in women (due to having less muscle mass). 24 hour urine creatinine normal range is 950 – 2,900 mg (8.4 – 29.6 mmol) for men and 600 – 1,700 mg (5.3 – 15.0 mmol) for women. Given a typical 24 hour urine production of 800-2000 ml, urine creatinine will range from about 2 to 32 nmol/µl. It is important that all sample readings are within the range of the standard curve. Samples should be diluted at least 10X for a 10 µl sample to be within the standard curve range.
- Creatine and sarcosine in samples will contribute to a possibly significant background signal. Samples should be run in duplicate with one of the pair used to determine the extent of the background which will be subtracted from the sample.

3. Initiate Reaction: Prepare sufficient Reaction Mix for the number of samples and standards to be run. Each well requires 50 µl of Reaction Mix containing:

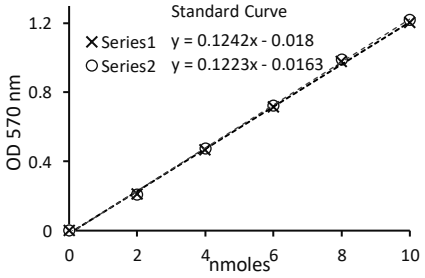
Reaction Mix	Absorbance based assay		Fluorescence based assay	
	Sample	Background Control	Sample	Background Control
Assay Buffer	42 µl	44 µl	43.6 µl	45.6 µl
Creatinase	2 µl	2 µl	2.0 µl	2.0 µl
Creatininase	2 µl	-----	2.0 µl	------
Sarcosine Oxidase/Peroxidase	2 µl	2 µl	2.0 µl	2.0 µl
ADHP Solution	2 µl	2 µl	0.4 µl	0.4 µl

Mix well. Add 50 µl of the appropriate Reaction Mix to each Standard and sample well, mix. Incubate at 37°C for 1 hr.

4. Measurement: Measure the absorbance at 570 nm or fluorescence using Excitation of 538 nm and emission of 587 nm.

5. Typical Results:

nmole Standard	Standard Raw Values		Background Corrected Values	
	OD			
0	0.0498	0.0473	0	0
2	0.2610	0.2575	0.2112	0.2102
4	0.5155	0.5212	0.4657	0.4739
6	0.7651	0.7699	0.7153	0.7226
8	1.026	1.0386	0.9762	0.9913
10	1.2535	1.2680	1.2037	1.2207



6. Calculation: Subtract 0 nmol standard from all other standards. Plot the Standard curve. Determine the slope of the standard curve. The slope of the standard curve defines the OD/nmol obtained from the assay. Correct the raw values for the test samples by subtracting the values for the paired background controls. Divide the background corrected test values by the slope of the standard curve to derive nmoles of Creatinine. Correct test samples for amount and dilution to determine the urea content of the original samples:

Total Creatinine of sample = (Total volume of test sample / volume of test sample applied to well) x creatinine in well

Creatinine concentration in Original Sample = Total creatinine of sample/ initial volume of sample diluted.

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