Product Information

Creatinine Colorimetric/Fluorometric Assay Kit

I. Kit Contents:

Components	K2130-100 100 assays	Cap Color	Part Number
Creatinine Assay Buffer	25 ml	WM	K2130-C-1
Creatinine Probe	0.2 ml	Red	K2130-C-2
Creatinase	1 vial	Blue	K2130-C-3
Creatininase	Lyophilized	Violet	K2130-C-4
Creatinine Enzyme Mix	Lyophilized	Green	K2130-C-5
Creatinine (10 µmol)	Lyophilized	Yellow	B1717

II. Introduction:

Creatinine is a product of creatine phosphate and is produced at a constant rate. Serum creatinine is used to determine glomerular filtration rate (GFR), which is an indicator of kidney function. Only when nephron is significantly damaged (> 75%), blood creatinine levels will increase. Creatinine clearance is used to standardize excretion of other compounds such as isoprostanes.

The Creatinine Colorimetric/Fluorometric Assay Kit provides a sensitive, fast and convenient way for accurate detection of creatinine levels in various biological fluids (serum, urine or CSF) based on colorimetric and fluorometric method. In the assay, creatinine is converted to creatine by creatininase, then creatine is converted to sarcosine, which is subsequently and specifically oxidized to generate a product which reacts with Creatinine Probe to yield fluorescence (Ex/Em = 538/587 nm) and red color (λmax = 570 nm). The kit is also suited for measurement of creatinine in serum/plasma, as well as in urine and other biological samples.

III. Application:

Measurement of creatinine in biological fluids.

IV. Sample Type:

Biological fluids: serum, urine, CSF etc.

V. User Supplied Reagents and Equipment:

96-well clear plate.

Multi-well spectrophotometer.

VI. Storage and Handling:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

VII. Reagent Preparation and Storage Conditions:

Creatinine Assay Buffer: Ready to use as supplied. It may be stored at 4°C or -20°C.

Creatinine Probe: Ready to use as supplied. Warm to room temperature to melt frozen DMSO prior to use. Store at -20°C, protect from light and moisture. Stable for at least 2 months.

Creatininase, Creatinase, Creatinine Enzyme Mix: Reconstitute with 220 µl of Assay Buffer. Keep on ice during use. Store at -20°C when not in use. Aliquot each and store until needed. Freeze/thaw should be limited to one time.

Creatinine Standard: Reconstitute with 100 µl of dH_2O to generate 100 mM Creatinine Standard. Dissolve completely. Store at -20°C, stable for 2 months.

VIII. Creatinine Assay Protocol:

1. Sample Preparation: Add 2 - 50 µl test samples to a 96-well plate. Adjust the volume to 50 µl/well with Creatinine Assay Buffer. Serum contains ~ 45 - 110 pmol/µl of creatinine.

Notes:

a. Creatinine concentrations can vary over a wide range depending on the sample. For unknown samples, we suggest doing pilot experiment & testing different dilutions to ensure the readings are in the linear range of the Standard Curve.

b. For samples having high protein content, we recommend deproteinizing the samples (tissue lysate or biological fluids) using 10kDa Spin Column . Add Sample to the spin column, centrifuge at 10,000 X g for 10 min. at 4 °C. Collect the filtrate.

c. Creatine in the sample will contribute to the background signal. If high creatinine levels are predicted in the sample, prepare parallel sample well(s) as sample background control(s).

d. Endogenous compounds in the sample may interfere with the assay, so it is suggested to spike samples with a known amount of Creatinine Standard (4 nmol) to ensure accurate determinations of creatinine in your sample.

2. Standard Curve Preparation: Mix 10 µl of Creatinine Standard with 990 µl of Assay Buffer to generate 1 nmol/µl Standard working solution. Add 0, 2, 4, 6, 8, 10 µl of the working solution to 6 consecutive wells. Bring the volume of each to 50 µl with Assay Buffer. If a more sensitive assay is desired, fluorescence can be utilized. Dilute the Standard working solution 10 fold, and follow the same procedure as for the colorimetric assay. Slightly better results are obtained with the fluorescent assay by diluting the probe 10X with DMSO.

3. Reaction Mix: Prepare enough reaction mix for the Standard and samples. For each well, prepare a total 50 µl Reaction Mix:

	Reaction Mix	Background Control Mix
Assay Buffer	42 µl	44 µl
Creatinase	2 µl	2 µl
Creatininase	2 µl	---
Enzyme Mix	2 µl	2 µl
Creatinine Probe	2 µl	2 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.

Note: Sarcosine and creatine generate background. If significant amounts of sarcosine or creatine are present in your samples, they can be measured by preparing a reaction without the creatininase (replace the 2 µl creatininase with 2 µl Assay Buffer) then the background can be subtracted from creatinine readings.

For the fluorescence assay, if the fluorescence background is too high, 0.4 µl of the probe can be used for each standard and samples, which will decrease the background reading significantly.

4. Measurement: Measure the absorbance at 570 nm or fluorescence at Ex/Em = 538/587 nm.

5. Calculation: Subtract 0 Standard reading from all readings. If sample background control reading is significant then subtract the sample background control reading from sample reading. Plot the Creatinine (CT) Standard Curve. For unspiked samples, apply the corrected OD to the Creatinine Standard Curve to get B nmol of creatinine in the sample well.

Sample Creatinine concentration (C) = B/V X D nmol/µl or mM.

Where: B is the amount of Creatinine in the sample well (nmol).

V is the sample volume added into the reaction well (µl).

D is the sample dilution factor.

Creatinine molecular weight: 113.12 g/mol.

Note: For spiked samples, correct for any sample interference by subtracting the sample reading from spiked sample reading.

For spiked samples, Creatinine amount in sample well (B) =(OD $_{\text{sample (corrected)}}$/ (OD $_{\text{sample + Creatinine Std(corrected)}}$ - OD $_{\text{sample (corrected)}}$))* Creatinine Spike (nmol).

Creatinine molecular weight: 113.12 g/mol.

Frequently Asked Questions:

1. What is the relation between lipid peroxidase and creatinine?

Creatinine is not directly related to lipid peroxidation. Isoprostane is used as a measure of lipid peroxidation. Creatinine is used for normalizing the Isoprostane concentration measured in different samples. Isoprostane/Creatinine ratio can be used as a measure for oxidative stress in samples.

2. If the standards turned brown instead of the usual purple color what is wrong?

The brown color reflects too much standard is used and the absorbance/fluorescence detector is saturated. Since the fluorometric assa is at least 10 times more sensitive, diluting the standard 1:100 as described in the datasheet should help resolve this issue. Also, the sensitivity of the fluorometer should be set at medium/low so that the detector does not get saturated easily.

3. Many samples read higher than the highest standard value in the fluorometric assay. 0.4 µl of the probe was used but it was not diluted.
The fluorometric assay is at least 10x more sensitive than the colorimetric assay. Is it essential to dilute the probe 10x and then use 0.4 µl of it to ensure the readings are not too high.

4. Can EDTA or Citrate used to prepare blood samples interfere in the assay?

Citrate should fine. But EDTA being a metal chelator could interfere in the function of the enzymes used for detection in this assay. We do not recommend EDTA for blood collection for any enzyme-based detection assay.

5. Is it essential to make a standard curve for every expt, or is one curve/kit enough?

Yes, it is strongly recommended to do the standards every time you do the expt. There is always a chance that something was done differently that day and we do not want any conditions to differ between standards and samples.

6. Can frozen samples be used with this assay?

Fresh samples are always preferred over frozen samples. However, frozen samples can also be used, provided, they were frozen right after isolation, were not freeze thawed multiple time (for which we recommend aliquoting the samples before freezing) and have been frozen for relatively short periods.

7. How are samples normalized against protein concentration?

A protein quantitation assay can be used with the supernatants from cell/tissue lysates or with any other liquid sample in the assay buffer.

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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