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Creatinine Colorimetric/Fluorometric Assay Kit

rev. 3/15

(Catalog # K625-100; 100 assays; Store at -20°C)

I. Introduction:

Creatinine is a breakdown product of creatine phosphate. Creatinine is produced and excreted at a constant rate, and blood creatinine is used to determine glomerular filtration rate (GFR), a measure of kidney function. Blood creatinine levels increase only in cases of significant (>75%) damage to nephrons. Creatinine clearance is frequently used as a means of standardizing excretion of other compounds such as isoprostanes. BioVision's Creatinine Assay Kit provides an accurate, convenient measure of creatinine concentration in biological fluids such as serum, urine or CSF. In the assay, creatinine is converted to creatine by creatininase, creatine is converted to sarcosine, which is specifically oxidized to produce a product which reacts with a probe to generate red color (OD 570 nm) and fluorescence (Ex/Em = 538/587 nm). Unlike the picric acid assay, this kit is suitable for serum/plasma creatinine determinations, as well as for urine and other biological samples.

Creatinine $\xrightarrow{\text{Creatininase}}$ Creatine $\xrightarrow{\text{Creatinase}}$ Sarcosine $\xrightarrow{\text{Oxidation}}$ Color and Fluorescence

II. Application:

- Measurement of creatinine in biological fluids.

III. Sample Type:

- Biological fluids: serum, urine, CSF etc.

IV. Kit Contents:

Components	K625-100	Cap Code	Part Number
Creatinine Assay Buffer	25 ml	WM	K625-100-1
Creatinine Probe	0.2 ml	Red	K625-100-2A
Creatinase	1 vial	Blue	K625-100-4
Creatininase	Lyophilized	Violet	K625-100-5
Creatinine Enzyme Mix	Lyophilized	Green	K625-100-6
Creatinine (10 µmol)	Lyophilized	Yellow	K625-100-7

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 (Cat # 1997). 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) = $\left(\frac{(\mathrm{OD}_{\text{sample (corrected)}})}{(\mathrm{OD}_{\text{sample + CT Std(corrected)}})\text{-}(\mathrm{OD}_{\text{sample(corrected)}})}\right) * Creatinine\ Spike\ (\mathrm{nmol})$

Creatinine molecular weight: 113.12 g/mol

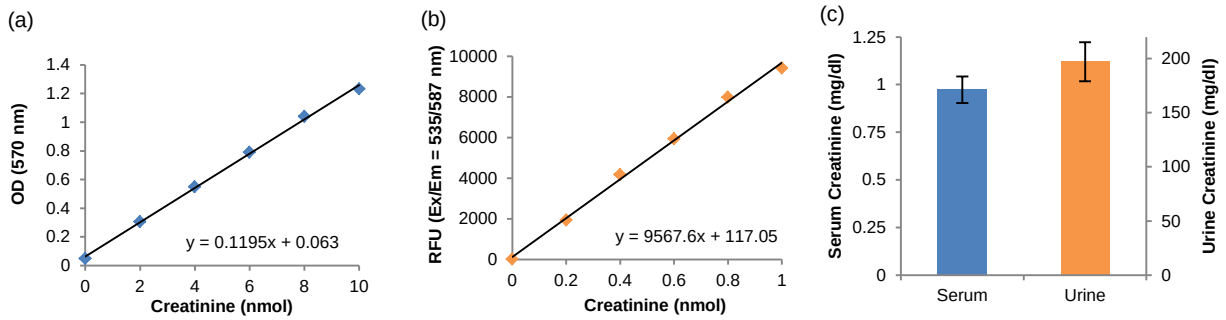

Figure. (a) Creatinine Standard Curve (colorimetric), (b) Creatinine Standard Curve (fluorometric). Fluorescence assay utilized 5X dilution of probe to reduce background. (c) Serum and urine samples were deproteinized using 10 kDa spin column (10000 x g, 10 min., 4°C) [Cat # 1997]. Sample filtrates (serum: 25 µl of 5-fold dilution; urine: 5 µl of 200-fold dilution) were spiked with known amounts of creatinine (serum: 0.6 nmol; urine: 4 nmol) and assayed according to kit protocol. Calculated concentrations: serum: 0.97 ± 0.06 mg/dl; Urine:197.1 ± 18 mg/dl.

IX. RELATED PRODUCTS:

Creatine Colorimetric/Fluorometric Assay Kit (K635)
Urea Colorimetric Assay Kit (K375)
Sarcosine Colorimetric/Fluorometric Assay Kit (K636)
10 kDa Spin Column (1997)

FOR RESEARCH USE ONLY! Not to be used on humans.