Creatinine Assay Kit

(Catalog #K625-100; 100 assays; Store Kit 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 (λ_{max} = 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 —Creatininase—> Creatine —Creatinase—> Sarcosine —Oxidation—> Color and Fluorescence

II. 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

III. Reconstitution of Reagents:

1. **Creatinine Assay Buffer:** Ready to use as supplied. It may be stored at 4°C, or -20°C
2. **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.
3. **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.
4. **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.

IV. Creatinine Assay Protocol:

1. **Prepare Standard:** 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 10-100 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.
2. **Prepare Samples:** High concentrations of protein may interfere with the assay. Samples containing protein may be filtered through a 10k MW cut-off filter (BioVision Cat. # 1997-25) prior to assay. Add 0-50 µl of sample to the wells and bring the volume to 50 µl with Assay buffer. **Note:** Serum contains ~45-110 pmol/µl of creatinine. For unknown samples, we suggest testing several different dilutions to ensure the readings are in the linear range of the standard curve.
3. **Prepare Reaction Mix:** Prepare enough reaction mix for the standard and samples. For each assay use:

	Samples	Background
Assay Buffer	42 µl	44 µl
Creatinase	2 µl	2 µl
Creatininase*	2 µl	-------
Enzyme Mix	2 µl	2 µl
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. **Read the plate** in a plate reader at 570 nm, or fluorescence with Ex/Em = 538/587 nm.

V. Calculations:

1. **Plot standard curve:** Subtract reagent background from all the readings. Plot readings vs. nmoles creatinine.
2. **Determine sample Creatinine concentrations:** Subtract sarcosine and creatine background from creatinine samples. Apply the creatinine reading to the standard curve.

C = Sa/Sv nmol/µl, or mM

Where **Sa** is the sample amount of unknown in nmol from your standard curve.
Sv is the sample volume added to the well in micro-litter.
Creatinine molecular weight: 113.12.

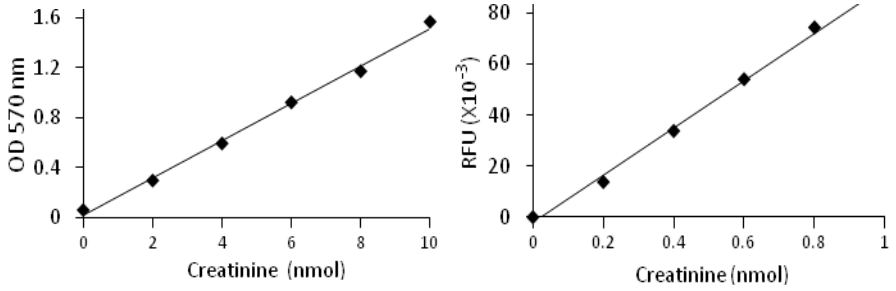

Figure 1: Creatinine Assay performed according to instructions. Fluorescent assay utilized 10X dilution of probe to reduce background

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

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GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Samples were not deproteinized (if indicated in datasheet)	• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed, deproteinize samples
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		