

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 creatine 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 ($\lambda_{\text{max}} = 570 \text{ nm}$) 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 $\xrightarrow{\text{Creatininase}}$ Sarcosine $\xrightarrow{\text{Oxidation}}$ Color and Fluorescence.

II. Kit Contents:

Components	K625-100	Cap Code	Part Number
Creatinine Assay Buffer	25 ml	WM	K625-100-1
Creatinine Probe	Lyophilized	Red	K625-100-2
DMSO (Anhydrous)	0.4 ml	Brown	K625-100-3
Creatinase	Lyophilized	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:** Dissolve in 220 μl DMSO (provided). Vortex to dissolve. Store at -20°C, protect from light and moisture. Stable for at least 2 months.
- 3. Creatinase, 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:
 - 42 μl Assay Buffer
 - 2 μl Creatinase
 - 2 μl Creatininase*
 - 2 μl Enzyme Mix
 - 2 μl probe**

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. nmol creatinine.

- 2. Determine sample Creatinine concentrations:** Subtract sarcosine and creatine background from creatinine samples. Apply the creatinine reading to the standard curve.

$$C = S_a/S_v \text{ nmol}/\mu\text{l}, \text{ or mM}$$

Where S_a is the sample amount of unknown in nmol from your standard curve.

S_v is the sample volume added to the well in micro-liter.

Creatinine molecular weight: 113.12.

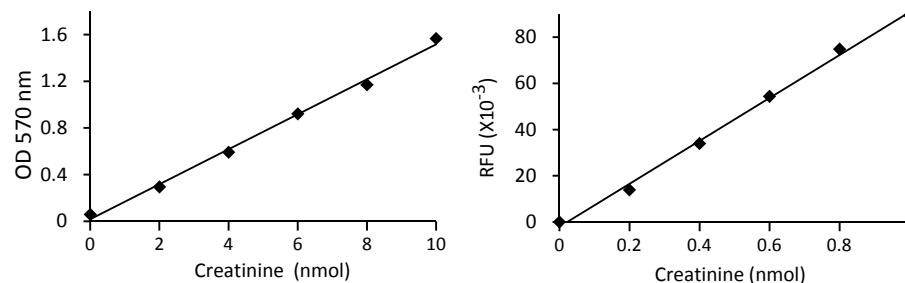


Figure 1: Creatinine Assay performed according to instructions. Fluorescent assay utilized 10X dilution of probe to reduce background