

Creatine Assay Kit

(Catalog #K635-100; 100 assays; Store Kit at -20°C)

I. Introduction:

Creatine is an endogenous compound whose function is to maintain a high ATP/ADP ratio, by way of its phosphorylated form and creatine kinase. Creatine supplementation has been used in the treatment of muscular, neurological and neurodegenerative diseases, as well as a sport performance enhancer. Detection of creatine level has wide applications in research and development. BioVision's Creatine Assay Kit provides an accurate, convenient measure of creatine in a variety of biological samples. In the assay, creatine is enzymatically converted to sarcosine which is then specifically oxidized to generate a product that converts a colorless probe to an intensely red color ($\lambda_{\text{max}} = 570 \text{ nm}$), and highly fluorescent (Ex/Em = 538/587 nm) product. Creatine is therefore easily detected by either colorimetric or fluorometric methods. Detection range 0.001 – 10 mM Creatine.

II. Kit Contents:

Components	K635-100	Cap Code	Part Number
Creatine Assay Buffer	25 ml	WM	K635-100-1
Creatine Probe	Lyophilized	Red	K635-100-2
DMSO (Anhydrous)	0.4 ml	Brown	K635-100-3
Creatinase	Lyophilized	Blue	K635-100-4
Creatine Enzyme mix	Lyophilized	Green	K635-100-5
Creatine Standard (10 μmol)	Lyophilized	Yellow	K635-100-6

III. Reconstitution of Reagents:

- Creatine Assay Buffer:** Ready to use as supplied. It may be stored at 4°C or -20°C.
- Creatine 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.
- Creatinase, Creatine 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.
- Creatine Standard:** Reconstitute with 100 μl of dH₂O to generate 100 nmol/ μl Creatine Standard. Dissolve completely. Store at -20°C, stable for 2 months.

IV. Assay Protocol:

- Prepare Standard:** Mix 10 μl reconstituted creatine standard with 990 μl of Assay Buffer, mix 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 to 50 μl each well with Assay Buffer.

If a more sensitive method is desired, fluorescence assay can be utilized. Further dilute the standard 10-100 fold, and follow the same procedure as for the colorimetric assay.

- Prepare Samples:** High concentrations of proteins may interfere with the assay. Samples containing proteins may be filtered through a 10k MW cut-off filter (BioVision Cat.# 10kc-20) prior to assay. Add 0-50 μl of sample to the wells and bring the volume to 50 μl with Assay buffer.

Note: For unknown samples, we suggest testing several different dilutions to ensure the readings are in the linear range of the standard curve.

- Prepare Reaction Mix:** Prepare enough reaction mix for the standard and samples. For each assay:

44 μl Assay Buffer
2 μl Creatinase*
2 μl enzyme
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 gives background for the assay. For samples which may contain a significant amount of sarcosine, do a background control. Prepare a reaction without the creatinase (replacing the creatinase with 2 μl assay buffer).

- Read the plate in a plate reader at 570 nm, or fluorescence at Ex/Em = 538/587 nm.

V. Calculations:

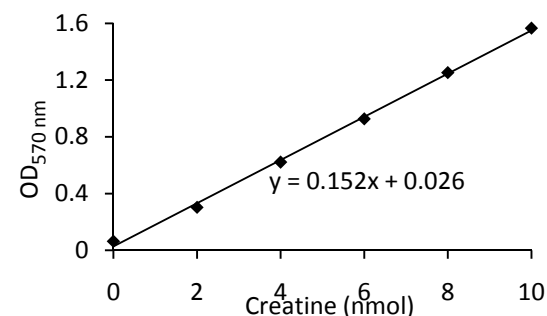
- Plot Standard Curve:** Subtract reagent background from all readings. Plot readings vs. nmoles creatine.
- Determine sample Creatine concentrations:** Subtract the background reading from the creatine assay sample. Apply the creatine reading to the standard curve. Creatine concentration:

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

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-litter.

Creatine Molecular Weight: 131.13.



Sarcosine Standard Curve: The assay is performed follow the kit procedure.

VI. Related Products:

Amino Acid Assay Kit	Glucose, Galactose, lactose, sucrose Assay Kit.
Sarcosine, Creatinine Assay Kit.	Glutamate Assay Kit
Glutathione Assay Kit	Choline Assay Kit