

## Creatine Colorimetric/Fluorometric 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 ( $\lambda_{max} = 570\text{nm}$ ), 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 ( in DMSO)	0.2 ml	Red	K635-100-2A
Creatinase	Lyophilized	Blue	K635-100-4
Creatine Enzyme mix	Lyophilized	Green	K635-100-5
Creatine Standard (10 $\mu\text{mol}$ )	Lyophilized	Yellow	K635-100-6

### III. Reconstitution of Reagents:

- 1. Creatine Assay Buffer:** Ready to use as supplied. It may be stored at 4°C or -20°C.
- 2. Creatine Probe:** ready to use as supplied. Warm to 18°C before use to melt frozen DMSO. Store at -20°C, protect from light and moisture. Stable for 2 months.
- 3. Creatinase, Creatine Enzyme mix:** Reconstitute with 220  $\mu\text{l}$  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. Creatine Standard:** Reconstitute with 100  $\mu\text{l}$  of dH<sub>2</sub>O to generate 100nmol/ $\mu\text{l}$  Creatine Standard. Dissolve completely. Store at -20°C, stable for 2 months.

### IV. Assay Protocol:

- 1. Prepare Standard:** Mix 10 $\mu\text{l}$  reconstituted creatine standard with 990  $\mu\text{l}$  of Assay Buffer, mix to generate 1nmol/ $\mu\text{l}$  standard working solution. Add 0, 2, 4, 6, 8, 10  $\mu\text{l}$  of the working solution to 6 consecutive wells. Bring the volume to 50  $\mu\text{l}$  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.

- 2. Prepare Samples:** High concentrations of proteins may interfere with the assay. Samples containing proteins may be filtered through a 10kDa MW cut-off filter (BioVision Cat. #1997-25) prior to assay. Add 0-50  $\mu\text{l}$  of sample to the wells and bring the volume to 50  $\mu\text{l}$  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.

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

44  $\mu\text{l}$  Assay Buffer  
 2  $\mu\text{l}$  Creatinase\*  
 2  $\mu\text{l}$  enzyme  
 2  $\mu\text{l}$  probe

- 4.** Mix well. Add 50  $\mu\text{l}$  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  $\mu\text{l}$  assay buffer).

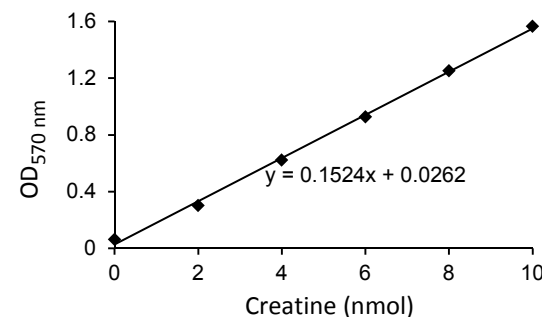
- 5.** Read the plate in a plate reader at 570 nm, or fluorescence at Ex/Em = 538/587nm.

### V. Calculations:

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

$$C = Sa/Sv \quad \text{nmol}/\mu\text{l, or mM}$$

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.  
 Creatine Molecular Weight: 131.13.



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

### 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

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

## GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> <li>• Use of ice-cold assay buffer</li> <li>• Omission of a step in the protocol</li> <li>• Plate read at incorrect wavelength</li> <li>• Use of a different 96-well plate</li> </ul>	<ul style="list-style-type: none"> <li>• Assay buffer must be at room temperature</li> <li>• Refer and follow the data sheet precisely</li> <li>• Check the wavelength in the data sheet and the filter settings of the instrument</li> <li>• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates</li> </ul>
Samples with erratic readings	<ul style="list-style-type: none"> <li>• Use of an incompatible sample type</li> <li>• Samples prepared in a different buffer</li> <li>• Samples were not deproteinized (if indicated in datasheet)</li> <li>• Cell/ tissue samples were not completely homogenized</li> <li>• Samples used after multiple free-thaw cycles</li> <li>• Presence of interfering substance in the sample</li> <li>• Use of old or inappropriately stored samples</li> </ul>	<ul style="list-style-type: none"> <li>• Refer data sheet for details about incompatible samples</li> <li>• Use the assay buffer provided in the kit or refer data sheet for instructions</li> <li>• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated</li> <li>• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope</li> <li>• Aliquot and freeze samples if needed to use multiple times</li> <li>• Troubleshoot if needed, deproteinize samples</li> <li>• Use fresh samples or store at correct temperatures till use</li> </ul>
Lower/ Higher readings in Samples and Standards	<ul style="list-style-type: none"> <li>• Improperly thawed components</li> <li>• Use of expired kit or improperly stored reagents</li> <li>• Allowing the reagents to sit for extended times on ice</li> <li>• Incorrect incubation times or temperatures</li> <li>• Incorrect volumes used</li> </ul>	<ul style="list-style-type: none"> <li>• Thaw all components completely and mix gently before use</li> <li>• Always check the expiry date and store the components appropriately</li> <li>• Always thaw and prepare fresh reaction mix before use</li> <li>• Refer datasheet &amp; verify correct incubation times and temperatures</li> <li>• Use calibrated pipettes and aliquot correctly</li> </ul>
Readings do not follow a linear pattern for Standard curve	<ul style="list-style-type: none"> <li>• Use of partially thawed components</li> <li>• Pipetting errors in the standard</li> <li>• Pipetting errors in the reaction mix</li> <li>• Air bubbles formed in well</li> <li>• Standard stock is at an incorrect concentration</li> <li>• Calculation errors</li> <li>• Substituting reagents from older kits/ lots</li> </ul>	<ul style="list-style-type: none"> <li>• Thaw and resuspend all components before preparing the reaction mix</li> <li>• Avoid pipetting small volumes</li> <li>• Prepare a master reaction mix whenever possible</li> <li>• Pipette gently against the wall of the tubes</li> <li>• Always refer the dilutions in the data sheet</li> <li>• Recheck calculations after referring the data sheet</li> <li>• Use fresh components from the same kit</li> </ul>
Unanticipated results	<ul style="list-style-type: none"> <li>• Measured at incorrect wavelength</li> <li>• Samples contain interfering substances</li> <li>• Use of incompatible sample type</li> <li>• Sample readings above/below the linear range</li> </ul>	<ul style="list-style-type: none"> <li>• Check the equipment and the filter setting</li> <li>• Troubleshoot if it interferes with the kit</li> <li>• Refer data sheet to check if sample is compatible with the kit or optimization is needed</li> <li>• Concentrate/ Dilute sample so as to be in the linear range</li> </ul>
<p><b>Note:</b> The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.</p>		