# 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 ($\lambda_{max}$ = 570nm), 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 µmol) | 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 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. **Creatine Standard:** Reconstitute with 100 µl of $dH_2O$ to generate 100nmol/µl Creatine Standard. Dissolve completely. Store at -20°C, stable for 2 months.

## IV. Assay Protocol:

1. **Prepare Standard:** Mix 10µl reconstituted creatine standard with 990 µl of Assay Buffer, mix to generate 1nmol/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.

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 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.

3. **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

4. 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).

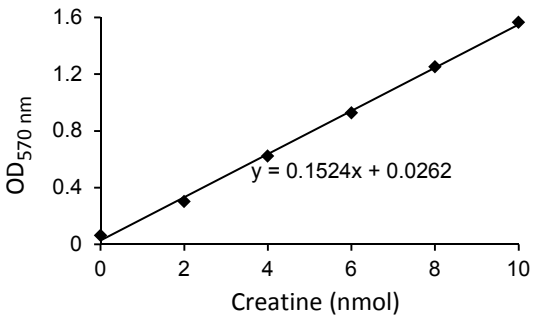
5. Read the plate in a plate reader at 570 nm, or fluorescence at Ex/Em = 538/587nm.

## 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 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.
   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 | • 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. | | |