ab65340- Creatinine Assay kit (Colorimetric/Fluorometric)

For the rapid, sensitive and accurate measurement of Creatinine in biological fluids.
This product is for research use only and is not intended for diagnostic use

Storage and Stability: Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.
Aliquot components in working volumes before storing at the recommended temperature.
Reconstituted components are stable for 2 months.

Materials Supplied:

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Assay Buffer 2	25 mL	-20°C	-20°C
OxiRed™ Probe	0.2 mL	-20°C	-20°C
Creatinase	1 vial	-20°C	-20°C
Creatininase	1 vial	-20°C	-20°C
Sarcosine Enzyme Mix	1 vial	-20°C	-20°C
Creatinine Standard	1 vial	-20°C	-20°C

PLEASE NOTE: Assay Buffer 2 was previously labelled as Assay Buffer II and Creatinine Assay Buffer, and OxiRed™ Probe as OxiRed Probe and Creatinine Probe (in DMSO). The composition has not changed.

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to perform this assay:

- Microplate reader capable of measuring absorbance at OD 570 nm or fluorescence at Ex/Em = 538/587 nm
- MilliQ water or other type of double distilled water (ddH2O)
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom (for colorimetric assay) / 96 well plate with clear flat bottom, preferably black (for fluorometric assay)
- 10 kD Spin Columns (ab93349): for deproteinization step in fluid samples

Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening

Creatinine Standard: Reconstitute Creatinine Standard in 100 µL of ddH2O to generate 100 mM Creatinine Standard. Aliquot standard so that you have enough volume to perform the desired number of assays. Store aliquots at -20°C. NOTE: Freeze/thaw should be limited to one cycle.

Assay Buffer 2: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

OxiRed™ Probe: Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 min to thaw the DMSO solution before use. NOTE: DMSO tends to be a solid when stored at -20°C, even when left at room temperature so it needs to melt for a few minutes at 37°C. Aliquot OxiRed™ Probe so that you have enough volume to perform the desired number of assays. Store aliquots at -20°C protected from light. Once the OxiRed™ Probe is thawed, use within two months.

Creatininase, Creatinase and Sarcosine Enzyme Mix: Reconstitute with 220 µL of Assay Buffer 2. Keep on ice during the assay. Aliquot creatininase so that you have enough volume to perform the desired number of assays. Store aliquots at -20°C. ***NOTE****: Freeze/thaw should be limited to one cycle*.

Standard Preparation: Always prepare a fresh set of standards for every use.
Discard the working standard dilutions after use as they do not store well.

1. Prepare a 1 nmoL/µL Creatinine standard by diluting 10 µL of 100 mM Creatinine Standard in 990 µL of Assay Buffer 2.

For colorimetric assay:

2. Using 1 nmoL/µL Creatinine standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes

For Fluorometric assay:

2. Prepare a 0.1 nmol/ul creatine standard by diluting 100 ul of the 1nmol/ul standard generated in step 1 in 900 ul of Assay Buffer 2.
3. Using 0.1 nmoL/µL Creatinine standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes

Standard #	Volume of Creatinine Standard (µL)	Assay Buffer 2 (µL)	Final Volume standard in well (µL)	End Conc Creatinine in well (nmol/well)	
				Colorimetric assay	Fluorometric assay
1	0	150	50	0	0
2	6	144	50	2	0.2
3	12	138	50	4	0.4
4	18	132	50	6	0.6
5	24	126	50	8	0.8
6	30	120	50	10	1

Each dilution has enough amount of standard to set up duplicate reading (2 x 50 µL).

Sample Preparation

We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze samples in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

Biological fluids (Serum, Urine and CSF): High concentrations of protein interfere with the assay. Fluid samples containing high levels of protein can be deproteinized with our 10 kD Spin column (ab93349) to deproteinize biological fluids. Add sample to the spin column, centrifuge at 10,000 x *g* for 10 min at 4°C. Collect the filtrate.

NOTE*: We suggest using different volumes of sample to ensure readings are within the Standard Curve range.*

Endogenous compounds in the sample may interfere with the assay, so it is suggested to spike samples with a known amount of Creatinine Standard (0 – 10 nmol) to ensure accurate determinations of creatinine in your sample.

Assay Procedure

- **Equilibrate all materials and prepared reagents to correct temperature prior to use.**
- **We recommended to assay all standards, controls and samples in duplicate.**
- **Prepare all reagents, working standards, and samples as directed in the previous sections.**

1. **Set up Reaction wells:**
   - Standard wells = 50 µL Standard dilutions
   - Sample wells = 2 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer 2).
   - Sample Background control wells = 2 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer 2).
   - Mix enough reagents for the number of assays (samples, standards and background control) to be performed.

Creatinine Reaction Mix (colorimetric/Fluorometric assay):

1. Prepare enough reaction mix for the Standard and samples. For each well, prepare a total 50 µl Reaction Mix:

	Reaction Mix	***Background Control Mix**
Assay Buffer 2	42 µl	44 µl
Creatinase	2 µl	2 µl
Creatininase*	2 µl	---
Sarcosine Enzyme Mix	2 µl	2 µl
OxiRed™ Probe**	2 µl	2 µl

2. 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 2) then the background can be subtracted from creatinine readings.

****Note:** For the fluorescence assay, if the fluorescence background is too high, 0.4 µl of the OxiRed™ Probe can be used for each standard and samples, which will decrease the background reading significantly. Please include this chart for the Reaction Mix:

- Measure output on a microplate reader at OD 570 nm for **Colorimetric assay**
- Measure output on a microplate reader at Ex/Em = 538/587 nm for **Fluorometric assay.**

Calculations

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

1. Average the duplicate reading for each standard and sample.
2. If the sample background control is significant, then subtract the sample background control from sample reading.
3. Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.
4. Plot the corrected absorbance values for each standard as a function of the final concentration of Creatinine.
5. Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).
6. Concentration of Creatinine in the test samples is calculated as:

$$Creatinine\ Concentration = \left(\frac{Sa}{Sv}\right) * D$$

Where:

Sa = amount of Creatinine in the sample well calculated from the standard curve (nmol).
Sv = sample volume added to the reaction well (µl).
D = sample dilution factor
Creatinine molecular weight: 113.12 g/mol

Troubleshooting

Problem	**Cause**	**Solution**
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimeters: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use provided protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer (increase 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
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so as to be in the linear range
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

FAQs

What is the relation between lipid peroxidase and creatinine?

Creatinine is not directly related to lipid peroxidation. Isoprostane is used as a measure of lipid peroxidation. Creatinine is used for normalizing the Isoprostane concentration measured in different samples. Isoprostane/ Creatinine ratio can be used as a measure for oxidative stress in samples.

What might be the issue if the standards turn brown instead of the usual purple color?

The brown color reflects too much standard is used and the absorbance/fluorescence detector is saturated. Since the fluorometric assay is at least 10 times more sensitive, diluting the standard 1:100 as described in the datasheet should help resolve this issue. Also, the sensitivity of the fluorometer should be set at medium/low so that the detector does not get saturated easily.

Many samples read higher than the highest standard value in the fluorometric assay. We used 0.4 µL of OxiRed™ Probe but we didn't diluted.

The fluorometric assay is at least 10x more sensitive than the colorimetric assay. It is essential to dilute the OxiRed™ Probe 10X and then use 0.4 µL of it to ensure the readings are not too high.

Can Creatinine levels from the same animal (rat) vary >10% in two different assays? Is this a stability issue?

Urine Creatinine is a very stable analyte. It is not known to aggregate like proteins can under storage. See: **http://www.clinchem.org/content/44/8/1759.full**

If the diet of this animal was changed or there was oxidative stress or starvation, this could change urine creatinine concentrations from the same animal. Also, pipetting/dilution errors can account for differences.

Can EDTA or Citrate used to prepare blood samples, interfere in the assay?

Citrate should be fine. But EDTA being a metal chelator could interfere in the function of the enzymes used for detection in this assay. We do not recommend EDTA for blood collection for any enzyme-based detection assay.

Technical Support

Copyright © 2025 Abcam. All Rights Reserved. The Abcam logo is a registered trademark. All information / detail is correct at time of going to print.

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)