ab65340
Creatinine Assay kit
(Colorimetric/Fluorometric)

Instructions for use:

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.

Version 13 Last Updated 10 January 2020
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INTRODUCTION

1. BACKGROUND
Creatinine Assay Kit (Colorimetric/Fluorometric) (ab65340) provides an accurate, convenient measure of creatinine 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.

Unlike picric acid-based assays, this kit is suitable for serum/plasma creatinine determinations, as well as for urine and other biological samples.

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.

Principle:

Creatininase → Creatinase → Oxidation

Creatinine → Creatine → Sarcosine → Color & Fluorescence
2. **ASSAY SUMMARY**

- **Standard preparation**
- **Sample preparation***
- **Add reaction mix and incubate for 60 minutes at 37°C**
- **Measure optical density (OD570 nm)**
  - Fluorescence (Ex/Em = 535/587 nm)

* Sample needs deproteinization
3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. 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.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**
5. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. MATERIALS SUPPLIED

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Storage Condition (Before Preparation)</th>
<th>Storage Condition (After Preparation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine Assay Buffer</td>
<td>25 mL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Creatinine Probe (in DMSO)</td>
<td>200 μL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Creatinase (Lyophilized)</td>
<td>1 vial</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Creatininase (Lyophilized)</td>
<td>1 vial</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Creatinine Enzyme Mix (Lyophilized)</td>
<td>1 vial</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>10 µmol Creatinine Standard (Lyophilized)</td>
<td>1 vial</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
</tbody>
</table>
7. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully 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
8. TECHNICAL HINTS

- This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.

- Avoid foaming or bubbles when mixing or reconstituting components.

- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.

- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.

- Samples which generate values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.

- Make sure you have the right type of plate for your detection method of choice.

- Make sure all necessary equipment is switched on and set at the appropriate temperature.
9. REAGENT PREPARATION

9.1. 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.

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

9.3. Creatinine 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 probe so that you have enough volume to perform the desired number of assays. Store aliquots at -20°C protected from light. Once the probe is thawed, use within two months.

9.4. Creatinase:
Reconstitute with 220 µL of Assay Buffer. Keep on ice during the assay. Aliquot creatinase 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.

9.5. Creatinase:
Reconstitute with 220 µL of Assay Buffer. Keep on ice during the assay. Aliquot creatinase 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.
9.6. **Creatinine Enzyme Mix:**

Reconstitute with 220 µL of Assay Buffer. Keep on ice during the assay. Aliquot enzyme 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.
10. 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.

10.1. For colorimetric assay:

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

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

<table>
<thead>
<tr>
<th>Standard #</th>
<th>Volume of Creatinine Standard (µL)</th>
<th>Assay Buffer (µL)</th>
<th>Final Volume standard in well (µL)</th>
<th>End Conc Creatinine in well (nmol/well)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>150</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>144</td>
<td>50</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>138</td>
<td>50</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>132</td>
<td>50</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>126</td>
<td>50</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>120</td>
<td>50</td>
<td>10</td>
</tr>
</tbody>
</table>

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

10.2. **For fluorometric assay:**

10.2.1. Prepare a 1 nmoL/µL Creatinine Standard by diluting 5 µL of 100 mM Creatinine Standard in 495 µL of Assay Buffer.

10.2.2. Prepare a 0.1 nmol/ul creatine standard by diluting 100 ul of the 1nmol/ul standard generated in step 10.2.1 in 900 ul of assay buffer.

10.2.3. Using 0.1 nmoL/µL Creatinine standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

<table>
<thead>
<tr>
<th>Standard #</th>
<th>Volume of Creatinine Standard (µL)</th>
<th>Assay Buffer (µL)</th>
<th>Final Volume standard in well (µL)</th>
<th>End Conc Creatinine in well (nmol/well)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>150</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>144</td>
<td>50</td>
<td>0.2</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>138</td>
<td>50</td>
<td>0.4</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>132</td>
<td>50</td>
<td>0.6</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>126</td>
<td>50</td>
<td>0.8</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>120</td>
<td>50</td>
<td>1</td>
</tr>
</tbody>
</table>

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

11. SAMPLE PREPARATION

General Sample Information

- 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.

11.1. 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

12. 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.

**NOTE:** Sarcosine and creatine in samples will interfere with the assay as they can generate high background. If you suspect your samples contain sarcosine or creatine, set up Sample Background Controls.

12.1. **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).

Sample Background control wells = 2 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer).

12.2. **Creatinine Reaction Mix (COLORIMETRIC ASSAY):**

12.2.1. Prepare 50 µL of Reaction Mix for each reaction. Mix enough reagents for the number of assays (samples and controls) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

\[ X \mu L \text{ component } x (\text{Number reactions} + 1) \]

<table>
<thead>
<tr>
<th>Component</th>
<th>Reaction Mix (µL)</th>
<th>Background Reaction Mix (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Buffer</td>
<td>42</td>
<td>44</td>
</tr>
<tr>
<td>Creatinase</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Creatininase</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Enzyme Mix</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Creatinine Probe</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>
ASSAY PROCEDURE

12.2.2. Add 50 µL of Reaction Mix into each standard and sample wells.

12.2.3. Add 50 µL of Background Reaction Mix into the Sample Background control wells.

12.2.4. Mix and incubate at 37°C for 1 hour.

12.2.5. Measure output on a microplate reader at OD 570 nm.

12.3. Creatinine Reaction Mix (FLUOROMETRIC ASSAY):

12.3.1. Prepare 50 µL of Reaction Mix for each reaction. Mix enough reagents for the number of assays (samples and controls) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

\[ X \mu L \text{ component} \times (\text{Number reactions} +1) \]

<table>
<thead>
<tr>
<th>Component</th>
<th>Reaction Mix (µL)</th>
<th>Background Reaction Mix (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Buffer</td>
<td>43.6</td>
<td>45.6</td>
</tr>
<tr>
<td>Creatinase</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Creatininase</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Enzyme Mix</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Creatinine Probe*</td>
<td>0.4</td>
<td>0.4</td>
</tr>
</tbody>
</table>

*NOTE: For the fluorometric assay, use 0.4 µL/well of the Probe to decrease the background readings, therefore increasing detection sensitivity.

12.3.2. Mix enough reagents for the number of assays (samples, standards and background control) to be performed.

12.4. Add 50 µL of Reaction Mix into each standard and sample well.

12.5. Add 50 µL of Background Reaction Mix into each Sample Background control well.

12.6. Mix and incubate at 37°C for 1 hour.

12.7. Measure output immediately on a microplate reader at OD 570 nm for colorimetric assay or at Ex/Em = 538/587 nm for fluorometric assay.
13. 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.

13.1. Average the duplicate reading for each standard and sample.

13.2. If the sample background control is significant, then subtract the sample background control from sample reading.

13.3. Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.4. Plot the corrected absorbance values for each standard as a function of the final concentration of Creatinine.

13.5. 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).

13.6. Concentration of Creatinine in the test samples is calculated as:

\[
Creatinine \ Concentration = \left( \frac{Sa}{Sv} \right) \ast D
\]

Where:

- \(Sa\) = amount of Creatinine in the sample well calculated from the standard curve (nmol).
- \(Sv\) = sample volume added to the reaction well (\(\mu\)L).
- \(D\) = sample dilution factor

Creatinine molecular weight: 113.12 g/mol
13.7. For the amount of Creatinine in sample well (Sa) for spiked samples, correct for any sample interference by subtracting the sample reading from spiked sample reading by the formula below, before calculating the creatinine concentration in the sample using equation under Step 13.6.

\[
Creatinine = \left( \frac{OD_{\text{sample}}}{OD_{\text{spiked sample}} - OD_{\text{sample}}} \right) \times \text{Creatinine Spiked (nmol)}
\]

Where:

- \( OD_{\text{sample}} \) = corrected OD of sample only
- \( OD_{\text{spiked sample}} \) = corrected OD of creatinine standard-spiked sample
- Creatinine spiked = the amount of creatinine standard spiked into the samples, in nmol
14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

Figure 1: Standard curve: mean of duplicates (+/- SD) with background reads subtracted

Figure 2: Standard curve: mean of duplicates (+/- SD) with background reads subtracted.
Figure 3: Serum and urine samples were deproteinized using a 10 kD spin column (10,000 x g, 10 min, 4°C). Sample filtrates (serum: 25 µL of 5-fold dilution; urine: 5 µL of 200-fold dilution) were spiked with known amounts of creatinine (serum: 0.6 nmol; urine: 4 nmol) and assayed according to kit protocol. Calculated concentrations: serum: 0.97 ± 0.06 mg/dl; urine: 197.1 ± 18 mg/dL.

Figure 4: Creatinine measured in mouse and human serum plotted against RFU. Samples were diluted 5-10 fold.
**Figure 5:** Creatinine measured in mouse and human urine plotted against RFU. Samples were diluted 400-800 fold.

**Figure 6:** Creatinine levels in filtered human urine were measured in the presence or absence of creatininase (background signal subtracted).
15. QUICK ASSAY PROCEDURE

NOTE: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Solubilize Creatinine standard, thaw Creatinine probe, prepare enzyme mix; get equipment ready.
- Prepare Creatinine standard dilution for your detection method: colorimetric [2 – 10 nmol/well] or fluorometric [0.2 – 1 nmol/well].
- Prepare samples (including deproteinization step) in optimal dilutions to fit standard curve readings.
- Set up plate in duplicate for standard (50 μL), samples (50 μL) and background wells (50 μL).
- Prepare a master mix for colorimetric or fluorometric Creatinine Reaction Mix and (if appropriate) a master mix for colorimetric or fluorometric Background Reaction Mix:

<table>
<thead>
<tr>
<th>Components</th>
<th>Colorimetric / Background Reaction Mix (μL)</th>
<th>Fluorometric / Background Reaction Mix (μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Buffer</td>
<td>42 / 44</td>
<td>43.6 / 45.6</td>
</tr>
<tr>
<td>Creatinase</td>
<td>2 / 2</td>
<td>2 / 2</td>
</tr>
<tr>
<td>Creatininase</td>
<td>2 / 0</td>
<td>2 / 0</td>
</tr>
<tr>
<td>Enzyme Mix</td>
<td>2 / 2</td>
<td>2 / 2</td>
</tr>
<tr>
<td>Creatinine Probe</td>
<td>2 / 2</td>
<td>0.4 / 0.4</td>
</tr>
</tbody>
</table>

- Add 50 μL of Reaction / Background Reaction Mix to respective wells.
- Incubate plate at 37°C for 1 hour protected from light.
- Measure plate immediately at OD 570 nm for colorimetric assay or Ex/Em= 538/587 nm for fluorometric assay.
16. TROUBLESHOOTING

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

- EDTA
- Endogenous creatine and sarcosine
- Creatinine binding proteins in sample

18. 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 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 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.
19. NOTES
UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

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