Creatinine (serum) detection kit
Catalog # 907-035

1x96 wells
For use with mammalian serum and plasma

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FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
**Introduction**

The Assay Designs® Creatinine (serum) detection kit is designed to quantitatively measure creatinine present in serum samples. Please read the complete kit insert before performing this assay.

Creatinine (2-amino-1-methyl-5H-imadazol-4-one) is a metabolite of phosphocreatine (p-creatine), a molecule used as a store for high-energy phosphate that can be utilized by tissues for the production of ATP\(^1\). Creatine either comes from the diet or synthesized from the amino acids arginine, glycine, and methionine. This occurs in the kidneys and liver, although other organ systems may be involved and species-specific differences may exist\(^2\). Creatine and p-creatine are converted non-enzymatically to the metabolite creatinine, which diffuses into the blood and is excreted by the kidneys. In vivo, this conversion appears to be irreversible and in vitro it is favored by higher temperatures and lower pH\(^2\). Creatinine forms spontaneously from p-creatine\(^3\). Under normal conditions, its formation occurs at a rate that is relatively constant and as intra-individual variation is <15% from day to day, creatinine is a useful tool for normalizing the levels of other molecules found in urine. Additionally altered creatinine levels may be associated with other conditions that result in decreased renal blood flow such as diabetes and cardiovascular disease\(^4-6\).

![Chemical Structure of Creatine and Phosphocreatine](image)

**Principle**

1. Samples and standards are added to the wells of a 96 well plate.
2. Creatinine Detection Reagent is added. The plate is read at 490 nm at 1 minute.
3. After incubating for an addition 6 minutes, the plate is read again at 490 nm.
**Materials Supplied**

1. **Clear Half Area Microtiter Plate**  
   Two plates of 96 wells, Catalog No. 80-2272  
   The plate is ready to use.

2. **Assay Diluent**  
   5 mL, Catalog No. 80-2274  
   A buffer containing detergent and preservative.

3. **Creatinine Standard**  
   100µL, Catalog No. 80-2273  
   Creatinine at 100 mg/dL in deionized water.

4. **Creatinine Detection Reagent**  
   20 mL, Catalog No. 80-1789  
   A solution containing reagents to detect creatinine.

**Storage**

The kit should be stored at 4°C. All kit components are stable at their recommended storage temperatures until the kit expiration date. Recommended storage temperatures do not necessarily reflect shipping conditions.

**Materials Needed but Not Supplied**

1. Deionized or distilled water.

2. Precision pipets for volumes between 5 µL and 1,000 µL.

3. Disposable beakers for diluting buffer concentrates.


5. Disposable polypropylene tubes.

6. Microplate reader capable reading optical density at 490 nm.

7. Software for converting optical density readings from the plate reader and carrying out four parameter logistic curve (4PL) fitting.
**Reagent Preparation**

**Creatinine Standard Preparation**

Label four glass test tubes as #1 through #4. Pipet 230 µL of water into tube #1 and 100 µL into tubes #2-#4. Carefully add 20 µL of the standard stock solution to tube #1 and vortex completely. Add 100 µL of tube #1 to tube #2 and vortex completely. Repeat these serial dilutions for tubes #3 and #4.

![Image of standard preparation](image)

Diluted standards should be used within 2 hours of preparation. The concentration of creatinine in tubes is labeled above.

Refer to the plate layout at the end of this document to determine the number of wells to be used.

**Sample Handling**

This assay has been validated with human, rabbit, sheep and mouse serum, EDTA plasma, and heparin plasma samples. The end user should evaluate recoveries of creatinine in other plasma and serum samples being used. For measuring creatinine in urine samples please refer to our website for Cat. #907-030.

Hemolyzed or lipemic samples should not be used with this kit. Hemolyzed samples have shown a decrease in creatinine concentration with increasing hemoglobin, whereas lipemic samples have been shown to yield artificially high creatinine concentrations. Please see Cat. #907-034 for a convenient kit to measure hemoglobin levels in whole blood.

All samples should be centrifuged for 15 minutes at 14,000 rpm in an Eppendorf type centrifuge prior to running in the assay.
**Assay Procedure**

Allow the kit reagents to come to room temperature for 30 minutes. We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine thiol concentrations. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit. Refer to the Assay Layout Sheet to determine the number of wells to be used. Cover unused wells tightly with a plate sealer. **DO NOT REUSE WELLS!**

1. Pipet 25 µL of water into the blank wells.
2. Pipet 25 µL of Assay Diluent to all wells used.
3. Pipet 25 µL of standards into the bottom of the appropriate wells.
4. Pipet 25 µL of samples into the bottom of the appropriate wells.
5. Observe wells, checking for bubbles. If bubbles are present, tap the plate gently to remove prior to addition of Reagent.
6. Pipet 100 µL of Creatinine Detection Reagent into each well.
7. Incubate at room temperature.
8. Read optical density at 490 nm after 1 minute.
9. At 7 minutes re-read the optical density at 490 nm.
**Calculation of Results**

Subtract the average Optical Density of the standards at 1 minute from the average Optical Density of the standards at 7 minute and plot the result (Average Delta OD) versus the creatinine concentration of the standards. Generate a linear regression line and use the equation, $y = mx + b$ ($y$=Average delta OD; $x$=Creatinine Concentration; $m$=slope and $b$= intercept) to calculate the concentrations in the unknown samples.

**SI Unit Conversion:** 1 mg/dL Creatinine = 0.088 mM Creatinine
Performance Characteristics

Linearity
Linearity was determined by taking two human serum samples, one with a low diluted creatinine level of 0.75 mg/dL and one with a higher level of 3.78 mg/dL and mixing them in ratios given below. The measured concentrations were compared to the expected values.

<table>
<thead>
<tr>
<th>Low Serum %</th>
<th>High Serum %</th>
<th>Expected Conc. mg/dL</th>
<th>Observed Conc. mg/dL</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>0%</td>
<td>0.75</td>
<td>0.75</td>
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<tr>
<td>80%</td>
<td>20%</td>
<td>1.36</td>
<td>1.44</td>
<td>106.2%</td>
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<td>60%</td>
<td>40%</td>
<td>1.96</td>
<td>2.03</td>
<td>103.5%</td>
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<td>40%</td>
<td>60%</td>
<td>2.57</td>
<td>2.61</td>
<td>101.6%</td>
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<td>20%</td>
<td>80%</td>
<td>3.17</td>
<td>3.12</td>
<td>98.3%</td>
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<tr>
<td>0%</td>
<td>100%</td>
<td>3.78</td>
<td>3.78</td>
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</tbody>
</table>

Mean Recovery 102.4%

\[ y = 0.9274x + 0.1995 \]
\[ R^2 = 0.9989 \]
**Intra Assay Precision**
Three serum samples were run in replicates of 20 in an assay. The mean and standard deviation of the calculated creatinine concentrations were:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Creatinine Conc. (mg/dL)</th>
<th>%CV</th>
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<tbody>
<tr>
<td>1</td>
<td>0.99</td>
<td>3.0</td>
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<tr>
<td>2</td>
<td>1.50</td>
<td>2.4</td>
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<tr>
<td>3</td>
<td>3.82</td>
<td>2.2</td>
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**Inter Assay Precision**
Three serum samples were run in duplicates in twenty-two assays run over four days by four operators. The mean and standard deviation of the calculated creatinine concentrations were:

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<th>Creatinine Conc. (mg/dL)</th>
<th>%CV</th>
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<tr>
<td>1</td>
<td>0.82</td>
<td>10.8</td>
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<td>2</td>
<td>1.20</td>
<td>17.7</td>
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<tr>
<td>3</td>
<td>3.51</td>
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**Sample Values**
Eleven serum samples from a variety of different species were tested in the assay. Values ranged from 0.78 to 1.45 mg/dL with an average of 1.00 mg/dL.

**Cross Reactivity and Interferants**
It is well known that some typical components of serum may interfere with the Jaffe reaction for creatinine measurement. A serum sample was spiked with varying concentrations of bilirubin and tested in the assay. Bilirubin level in normal serum is between 0.2 and 1.0 mg/dL. The unspiked sample read at 0.86 mg/dL. No significant change to the measured creatinine level was seen up to an additional 1.0 mg/dL of bilirubin.
References

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**Limited Warranty**

Assay Designs, Inc. warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

Assay Designs must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if Assay Designs is not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

**Kits for Charity™**

Assay Designs has always been an active contributor to a number of local, national and international charities. We have broadened our charitable contributions by implementing a program called Kits for Charity™.

Each quarter, Assay Designs will feature a different non-religious and non-political charitable organization on our website. For each kit sold during this time period, we will make a monetary contribution to the featured charity. Please check our website for the current quarter’s charity to see what organization your purchases are helping to support.

If you have any suggestions for future Kits for Charity™ recipients, please contact us at 800.833.8651 or 734.668.6113.

**Contact Us**

For more details concerning the information within this kit insert, or to order any of the Assay Designs’ products, please call (734) 668-6113 between 8:30 a.m. and 5:30 p.m. EST. Orders or technical questions can also be transmitted by fax or e-mail 24 hours a day.