Creatinine (serum) Colorimetric Assay Kit

Item No. 700460

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1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a 4°C kit. For best results, store the kit as supplied or remove components and store as stated below.

Item Number	Item	Quantity/Size	Storage
10005314	Creatinine Standard	1 vial/3 ml	4°C
700461	Creatinine (serum) Color Reagent	2 vials/12 ml	RT
400508	Creatinine Alkaline Buffer	2 vials/5 ml	RT
10008477	Creatinine Sodium Borate	2 vials/2.5 ml	RT
10008478	Creatinine Surfactant	2 vials/7.5 ml	RT
400014	96-Well Solid Plate (Colorimetric Assay)	2 plates	RT
400012	96-Well Cover Sheet	2 ea	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

It is recommended to take appropriate precautions when using the kit reagents (*i.e.*, lab coat, gloves, eye goggles, etc.) as some of them may be harmful.

The sodium hydroxide and acid solutions are corrosive and harmful if swallowed. Contact with skin may cause burns. In case of contact with skin or eyes, rinse immediately with plenty of water for 15 minutes.

The color solution is harmful if swallowed and irritating to eyes, respiratory system, and skin. In case of contact with skin or eyes, rinse immediately with plenty of water for 15 minutes. The color solution is explosive when dry.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as specified in the **Materials Supplied** section, on page 3, and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance between 490-500 nm
2. Adjustable pipettes and a repeating pipettor
3. A source of pure water; glass distilled water or pure water is acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*

INTRODUCTION

Background

Creatine, occurring either from arginine and glycine synthesis in the kidney, liver, and pancreas, or from dietary intake, is transported in blood to the brain and muscle tissue where it is phosphorylated to phosphocreatine. Free creatine and phosphocreatine in muscle is converted non-enzymatically to creatinine which diffuses into the blood and then is excreted by the kidneys. Because the kidneys are responsible for clearing creatinine from the blood, measurement of serum creatinine levels is a useful indicator of renal function.[1] While creatinine levels vary depending on the severity of failure, the creatinine level of patients suffering from renal failure typically begins at approximately 2 mg/dl. In addition, abnormal creatinine levels have been implicated in diabetes, cardiovascular, and circulatory diseases.

About This Assay

Cayman's Creatinine (serum) Colorimetric Assay can be used to measure creatinine levels in plasma and serum. The assay relies on the Jaffe' reaction, wherein a yellow/orange color forms when the metabolite is treated with alkaline picrate.[2] The rate of color development is directly proportional to the concentration of creatinine in the sample and is measured at an absorbance between 490-500 nm. The kinetic nature of the assay eliminates interference from extraneous serum contaminants, such as lipids and bilirubin.[1,3]

PRE-ASSAY PREPARATION

Reagent Preparation

1. **Creatinine Standard - (Item No. 10005314)**

   The Creatinine Standard contains 3 ml of 20 mg/dl creatinine in water. It is ready to use to prepare the standard curve.

2. **Creatinine (serum) Color Reagent - (Item No. 700461)**

   Each vial contains 12 ml of 1.2% picric acid. It is ready to be used as supplied.

3. **Creatinine Alkaline Buffer - (Item No. 400508)**

   Each vial contains 5 ml of buffer. It is ready to be used as supplied.

4. **Creatinine Sodium Borate - (Item No. 10008477)**

   Each vial contains 2.5 ml of a sodium borate solution. It is ready to be used as supplied.

5. **Creatinine Surfactant - (Item No. 10008478)**

   Each vial contains 7.5 ml of a surfactant solution. It is ready to be used as supplied.

6. **Creatinine Reaction Buffer**

   The volume of Creatinine Reaction Buffer needed is dependent on the number of wells being assayed (calculate 100 µl for each well). To prepare sufficient reagent for one 96-well plate, mix together 2 ml Creatinine Sodium Borate, 6 ml Creatinine Surfactant, and 4 ml Creatinine Alkaline Buffer. The Creatinine Reaction Buffer is stable for one week when stored at room temperature.

Sample Preparation

Plasma

1. Collect blood using an anticoagulant such as heparin or citrate.
2. Centrifuge the blood at 700-1,000 x g for 10 minutes at 4°C. Pipette off the top yellow plasma layer without disturbing the white buffy layer. Store plasma on ice until assaying or freeze at -80°C. The plasma sample will be stable for one month. Repeated freeze/thaw cycles should be avoided.
3. Plasma does not need to be diluted before assaying. Typical creatinine concentrations in plasma range from 0.5-1.5 mg/dl.[4]

Serum

1. Collect blood without using an anticoagulant such as heparin or citrate.
2. Allow blood to clot for 30 minutes at 25°C.
3. Centrifuge the blood at 2,000 x g for 15 minutes at 4°C. Pipette off the top yellow serum layer without disturbing the white buffy layer. Store serum on ice. If not assaying the same day, freeze at -80°C. The sample will be stable for one month. Repeated freeze/thaw cycles should be avoided.
4. Serum does not need to be diluted before assaying. Typical creatinine concentrations in serum range from 0.5-1.5 mg/dl.[4]

Sample Matrix Properties

Linearity

Plasma and serum samples were spiked with creatinine to the final concentration of 7.5 mg/dl, serially diluted with Creatinine Reaction Buffer and tested in the assay. The results are shown below.

Dilution	Measured Concentration (mg/dl)	% Recovery
Plasma EDTA		
1:1	8.67	100.00
1:2	9.59	110.57
1:4	9.05	104.36
Serum		
1:1	7.91	100.00
1:4	8.58	108.40
1:8	8.85	111.90
1:16	7.96	100.65

Table 1. Linearity of plasma and serum samples

Spike and Recovery

Plasma and serum samples were spiked with different amounts of creatinine, diluted with Creatinine Reaction Buffer and evaluated with the assay. Results are shown below. The error bars represent standard deviations between several dilutions of each sample.

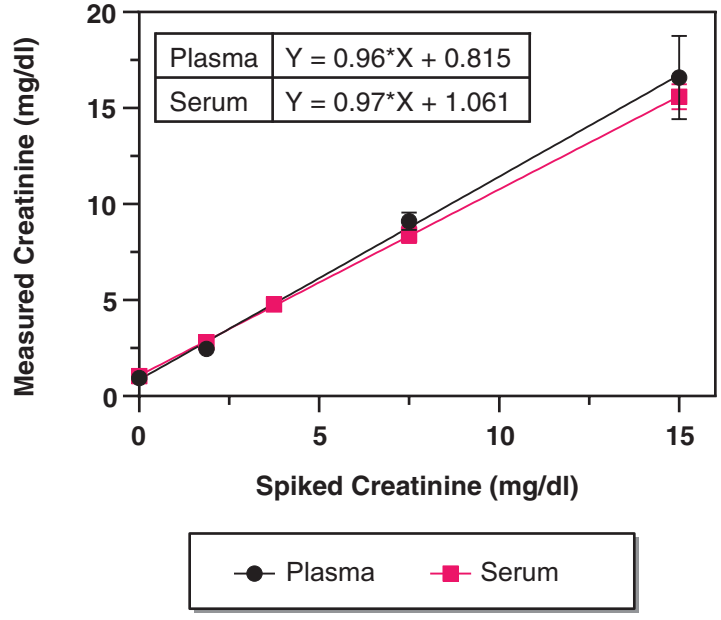
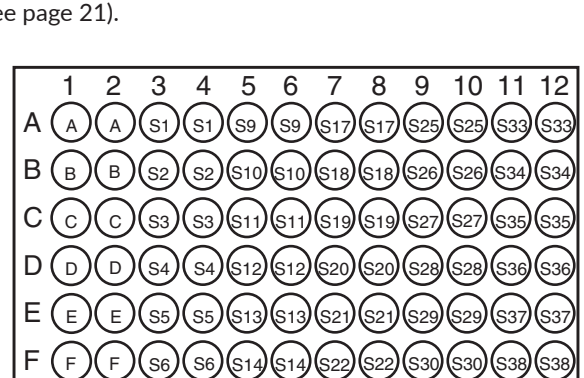


Figure 1. Spike and recovery of creatinine in plasma and serum samples

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. A typical layout of Creatinine Standards and samples to be measured in duplicate is given below in Figure 2. We suggest you record the contents of each well on the template sheet provided (see page 21).



A-H - Standard wells
S1-S40 - Sample wells

Figure 2. Sample plate format

Pipetting Hints

- It is recommended that a repeating pipettor be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 215 µl in all the wells.
- All reagents must be equilibrated to room temperature before beginning the assay.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended that the standards and samples be assayed at least in duplicate (triplicate is recommended).
- Twenty-six samples can be assayed in triplicate or forty in duplicate.
- The assay is performed at room temperature.
- Monitor the absorbance at 490-500 nm.

Standard Preparation

For the determination of creatinine in plasma or serum, prepare the Creatinine Standards according to Table 2. Take eight clean glass test tubes and label them A-H. Add the amount of Creatinine Standard (20 mg/dl) and pure water to each tube as described in Table 2.

Tube	Creatinine Standard (µl)	Pure Water (µl)	Final Concentration (mg/dl creatinine)
A	0	500	0
B	12.5	487.5	0.5
C	25	475	1
D	37.5	462.5	1.5
E	50	450	2.0
F	75	425	3.0
G	100	400	4.0
H	125	375	5.0

Table 2. Preparation of creatinine standards

Performing the Assay

1. **Standard Wells -** add 15 µl of Creatinine Standard (tubes A-H) per well in the designated wells on the plate (see **Sample plate format**, Figure 2, on page 12).
2. **Sample Wells -** add 15 µl of each sample to at least two wells.
3. Add 100 µl of Creatinine Reaction Buffer to all the wells being used.
4. Add 100 µl of Creatinine (serum) Color Reagent to all the wells being used and immediately start timing the reaction.
5. Immediately read and record the aborbance at 490-500 nm.
6. Continue incubating the plate at room temperature.
7. At seven minutes, read and record the aborbance at 490-500 nm.

ANALYSIS

Calculations

1. Calculate the ΔO.D. (optical density) by subtracting the initial (0 minute) absorbance reading from the final (7 minute) absorbance reading.

$$\Delta O.D. = A_{490}\,(7\text{ min}) - A_{490}\,(0\text{ min})$$

2. Subtract the average ΔO.D of standard A from itself and all other standards and samples. This is the adjusted ΔO.D.
3. Plot the adjusted ΔO.D of the standards (from step 2 above) as a function of the final concentration of creatinine from Table 2. See Figure 3, on page 17, for typical standard curve.
4. Calculate the creatinine concentration of the samples using the equation obtained from the linear regression of the standard curve, substituting adjusted ΔO.D. values for each sample.

$$\text{Creatinine (mg/dl)} = \left[\frac{\text{Adjusted sample } \Delta O.D. - (\text{y-intercept})}{\text{Slope}}\right] \times \text{Sample dilution (if needed)}$$

NOTE: To convert the results from mg/dl to µmol/l, multiply the creatinine concentration (mg/dl) by 88.4.

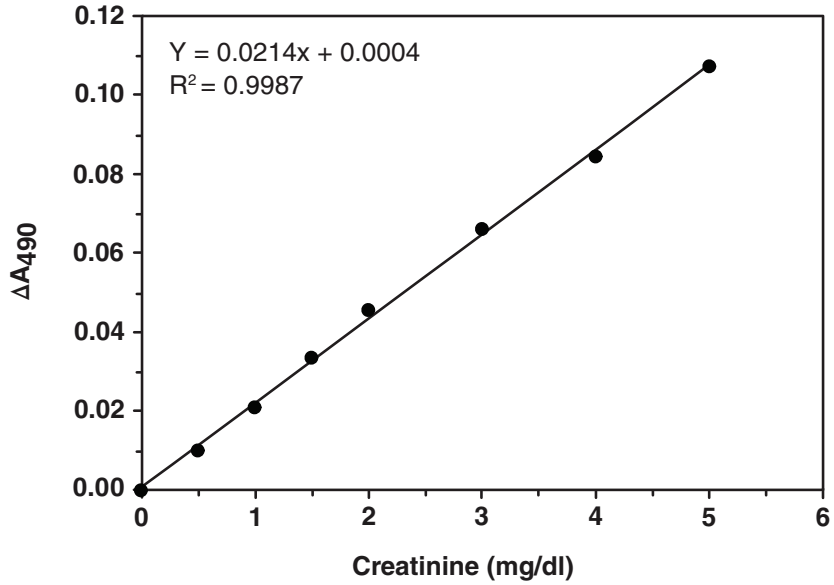


Figure 3. Typical creatinine standard curve

Performance Characteristics

Sensitivity:

The limit of detection (LOD) for this assay is approximately 0.5 mg/dl.

The lower limit of quantification (LLOQ) for this assay is approximately 1 mg/dl.

Precision:

When a series of 24 serum measurements were performed on the same day under the same experimental conditions, the intra-assay coefficient of variation was 18.5%. When a series of six serum measurements were performed on different days under the same experimental conditions, the inter-assay coefficient of variation was 5.6%.

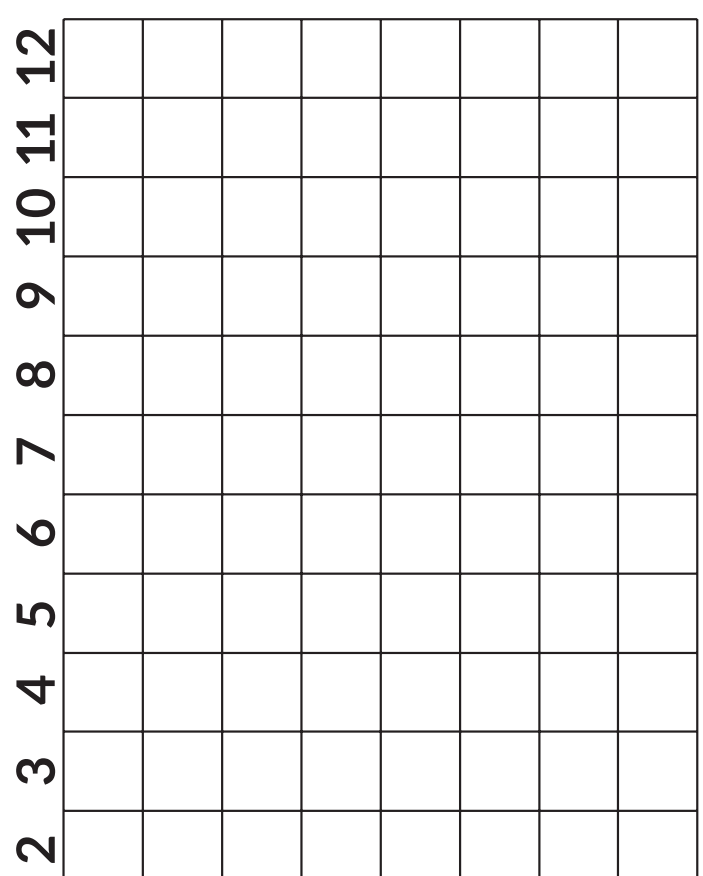
RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/ triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
No creatinine was detected in the sample well	A. One of the reagents were not added to the well B. The sample was too dilute or the concentration was below the detection limit of the assay	A. Re-assay the sample making sure to add Creatinine Reaction Buffer and Creatinine (serum) Color Reagent to the well B. Analyze sample without dilution (if applicable)
Sample absorbance values are too high	The concentration of creatinine in the sample is too high	Dilute samples with pure water to fall within the range of the standard curve
The creatinine standard curve did not develop properly	A. The creatinine standards were not diluted properly B. The standard has deteriorated C. One of the reagents was not added to the well	Set up the standards according to Table 1 on page 10 and re-assay

References

1. Bowers, L.D. and Wong, E.T. Kinetic serum creatinine assays. II. A critical evaluation and review. *Clin. Chem.* **26(5)**, 555-561 (1980).
2. Slot, C. Plasma creatinine determination. A new and specific Jaffe reaction method. *Scand. J. Clin. Lab. Invest.* **17**, 381-387 (1965).
3. Bowers, LD. Kinetic serum creatinine assays I. The role of various factors in determining specificity. *Clin. Chem.* **26(5)**, 551-554 (1980)
4. Barret, E. and Addis, T. The serum creatinine concentration of normal individuals. *J Clin Invest.* **26(5)**, 875-878 (1947)



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.