Creatinine (urinary) Colorimetric Assay Kit

Item No. 500701

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	96 wells Quantity/Size	480 wells Quantity/Size
10005314	Creatinine Standard	1 vial/3 ml	1 vial/15 ml
10005315	Creatinine Color Reagent	1 vial/12 ml	1 vial/60 ml
10005316	Creatinine Sodium Hydroxide	1 vial/5 ml	1 vial/25 ml
10005317	Creatinine Acid Solution	1 vial/1 ml	1 vial/5 ml
10008477	Creatinine Sodium Borate	1 vial/2.5 ml	1 vial/12.5 ml
10008478	Creatinine Surfactant	1 vial/7.5 ml	1 vial/37.5 ml
400014	96-Well Solid Plate (Colorimetric Assay)	1 plate	5 plates
400012	96-Well Cover Sheet	1 cover	5 covers

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

Store the Creatinine Standard at 4°C and the rest of the kit at room temperature (18-26°C). This kit will perform as specified if stored properly 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. An orbital microplate shaker
3. Adjustable pipettes and a repeating pipettor
4. A source of pure water; glass-distilled or HPLC-grade water is acceptable

INTRODUCTION

Background

Creatinine is a degradation product of creatine and phosphocreatine, which are involved in the creatine kinase reaction that is a source of phosphate for the conversion of ADP to ATP for energy in cells and tissues that consume ATP rapidly, such as skeletal muscle and the brain.[1] Muscle creatine and phosphocreatine are converted nonenzymatically and irreversibly at a steady rate to creatinine, which is transported to the kidney in the blood and excreted in the urine.[1,2] The amount of creatinine produced is proportional to an individual's muscle mass and can be used to normalize data in assays using urine samples.[2,3] It has also been used as a measure of the glomerular filtration rate (GFR) to assess renal function, estimate and monitor the extent of functional renal impairment, and detect renal disease.[1,3]

About This Assay

Cayman's Creatinine (urinary) Colorimetric Assay Kit can be used to measure creatinine levels in urine. The assay relies on the Jaffe reaction, wherein a yellow/orange color forms when the metabolite is treated with alkaline picrate.[4] The color derived from creatinine is then destroyed at acidic pH. The difference in color intensity measured at 500 nm before and after acidification is proportional to the creatinine concentration.[3,5,6] The sample creatinine concentration is determined using a creatinine standard curve with a range of 0-15 mg/dl.

PRE-ASSAY PREPARATION

Reagent Preparation

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

   The Creatinine Standard contains 20 mg/dl of creatinine in water. It is ready to use to prepare the standard curve. Sufficient Creatinine Standard is provided to prepare two standard curves using the 3 ml size or ten standard curves using the 15 ml size.

2. **Creatinine Color Reagent - (Item No. 10005315)**

   The color reagent contains 1.2% picric acid. The picric acid may contain crystals. This is normal and will disappear upon making the Alkaline Picrate Solution.

3. **Creatinine Sodium Hydroxide - (Item No. 10005316)**

   The vial contains 1 M sodium hydroxide (NaOH). It is ready to use as supplied.

4. **Creatinine Acid Solution - (Item No. 10005317)**

   The acid solution contains a mixture of sulfuric and acetic acid. It is ready to use as supplied.

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

   The vial contains a solution of sodium borate. It is ready to use as supplied.

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

   The vial contains a solution of surfactant. It is ready to used as supplied.

7. **Alkaline Picrate Solution**

   The volume of Alkaline Picrate Solution needed is dependent on the number of wells being assayed. Calculate 150 µl for each well (*i.e.*, To prepare sufficient reagent for one 96-well plate, mix together 2 ml of Creatinine Sodium Borate, 6 ml of Creatinine Surfactant, 10 ml of Creatinine Color Reagent, and 3.6 ml of Creatinine NaOH). The Alkaline Picrate Solution is stable for at least one week stored in the dark at room temperature.

Sample Preparation

Urine

Typically, human urine has creatinine levels in the range of 25-400 mg/dl (one-time collection) or 500-2,000 mg/24 hours.

1. Collect urine in a clean container and store on ice. If not assaying on the same day, freeze the sample at -80°C. The sample will be stable for at least one month.
2. If a 24-hour urine sample is desired, collect the total volume of urine over a 24-hour period. Store the pooled urine at 4°C until all the collections are taken. If not assaying after all the collections are taken, freeze 5 ml of the pooled 24-hour collection at -80°C. The sample will be stable for at least one month.
3. Urine should be diluted 1:10 or 1:20 with pure water before assaying.

NOTE: The Creatinine (urinary) Colorimetric Assay is not recommended for plasma or serum samples. Precipitation may occur in the wells upon the addition of the acid solution.

Sample Matrix Properties

Spike and Recovery

Human urine was spiked with different amounts of Creatinine Standard, serially diluted with water, and evaluated using the Creatinine (urinary) Colorimetric Assay Kit. The results are shown below. The error bars represent standard deviations obtained from two or three dilutions of the same sample.

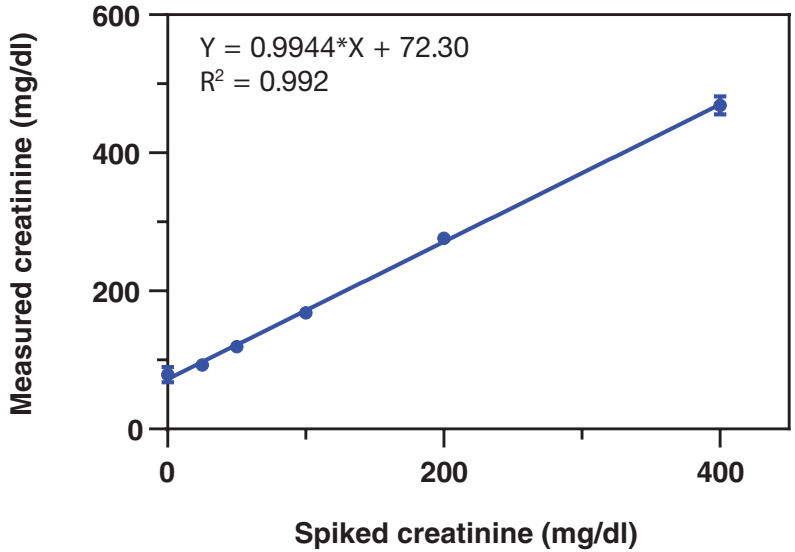


Figure 1. Spike and recovery of creatinine in urine

Linearity

Urine was serially diluted with water and evaluated for linearity using the Creatinine (urinary) Colorimetric Assay Kit.

Dilution Factor	Measured Concentration (mg/dl)	Linearity (%)
Human urine, sample 1		
10	255.06	100
15	284.57	112
20	262.95	103
30	269.40	106
Human urine, sample 2		
10	83.61	100
15	91.75	110
20	86.82	104
30	87.40	105
Human urine, sample 2		
10	18.27	100
15	18.50	101
20	17.79	97
30	17.59	96

Table 1. Linearity in human urine

*NOTE: Linearity has been calculated using the following formula: %Linearity = (Observed concentration value, dilution adjusted / First observed concentration value in the dilution series, dilution adjusted)*100*

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, below. We suggest you record the contents of each well on the template sheet provided (see page 21).

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33
B	B	B	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
C	C	C	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
D	D	D	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
E	E	E	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
F	F	F	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
G	G	G	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
H	H	H	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40

A-H = Standards
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 to 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 170 µl in all wells.
- All reagents except samples 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.
- If the concentration of creatinine in the sample is not known or if it is expected to be beyond the range of the standard curve, it is prudent to assay the sample at several dilutions.
- It is recommended that the standards and samples be assayed at least in duplicate (triplicate is recommended).
- Monitor the absorbance at 490-500 nm using a plate reader.

Standard Preparation

For the determination of creatinine in urine, prepare the Creatinine Standards according to Table 2, below. Take eight clean glass test tubes and label them A-H. Add the amount of Creatinine Standard and pure water to each tube as described in Table 2, below.

Tube	Creatinine Standard (µl)	Pure water (µl)	Final concentration (mg/dl creatinine)
A	0	500	0
B	50	450	2
C	100	400	4
D	150	350	6
E	200	300	8
F	250	250	10
G	300	200	12
H	375	125	15

Table 2. Concentration of standards

Performing the Assay

1. **Creatinine Standard Wells -** Add 15 µl of Creatinine Standard (tubes A-H) per well in the designated wells on the plate (see suggested plate configuration, Figure 2, page 12).
2. **Sample Wells -** Add 15 µl of sample to two wells. To obtain reproducible results, creatinine levels from each sample should fall within the absorbance values of the standard curve. When necessary, samples can be diluted with pure water to bring the creatinine concentration to this level.
3. Initiate the reactions by adding 150 µl of Alkaline Picrate Solution to all the wells being used.
4. Cover the plate with the plate cover and incubate on a shaker for 10 minutes at room temperature.
5. Remove the plate cover and read the absorbance at 490-500 nm using a plate reader. This absorbance is the initial absorbance reading.
6. Add 5 µl of acid solution to all of the wells being used.
7. Cover the plate with the plate cover and incubate on a shaker for 20 minutes at room temperature.
8. Remove the cover and read the absorbance at 490-500 nm using a plate reader. This absorbance is the final absorbance reading.

ANALYSIS

Calculations

1. Calculate the average initial absorbance of each standard and sample.
2. Calculate the average final absorbance of each standard and sample.
3. Subtract the average final absorbance from the average initial absorbance. This is your corrected absorbance.
4. Subtract the average corrected absorbance of standard A from itself and all other standards and samples. This is the adjusted absorbance.
5. Plot the adjusted absorbance of the standards (from step 4 above) as a function of the final concentration of creatinine from Table 2 (on page 14). See Figure 3, on page 18, for a typical standard curve.
6. Calculate the creatinine concentration of the samples using the equation obtained from the linear regression of the standard curve substituting adjusted absorbance values for each sample.

$$\text{Creatinine (mg/dl)} = \left[\frac{\text{Sample absorbance - (y-intercept)}}{\text{Slope}}\right] \times \text{Sample dilution}$$

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

Performance Characteristics

Precision:

Intra-assay coefficient of variation = 2.7% (n = 84). Inter-assay coefficient of variation = 3% (n = 5).

Lower Limit of Detection (LLOD):

The LLOD for this assay is 0.06 mg/dl.

Lower Limit of Quantification (LLOQ):

The LLOQ for this assay is 0.5 mg/dl.

Representative Standard Curve

The standard curve presented below is an example of the data typically produced with this kit; however, your results will not be identical to these. You must run a new standard curve - do not use these to determine the values of your samples.

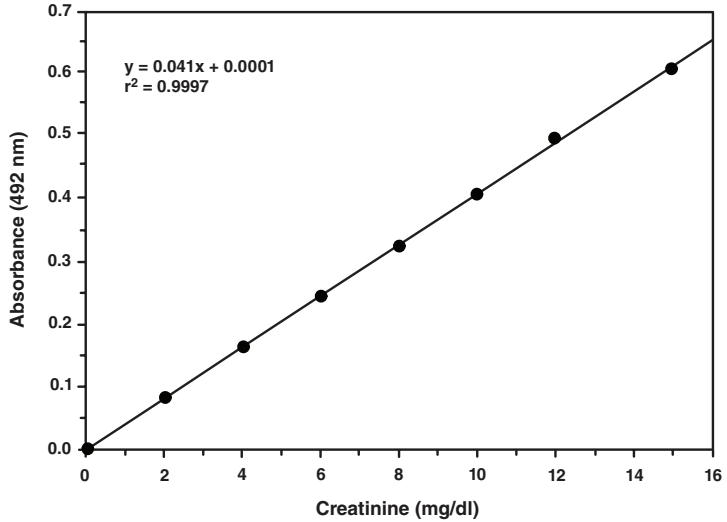


Figure 3. Creatinine standard curve

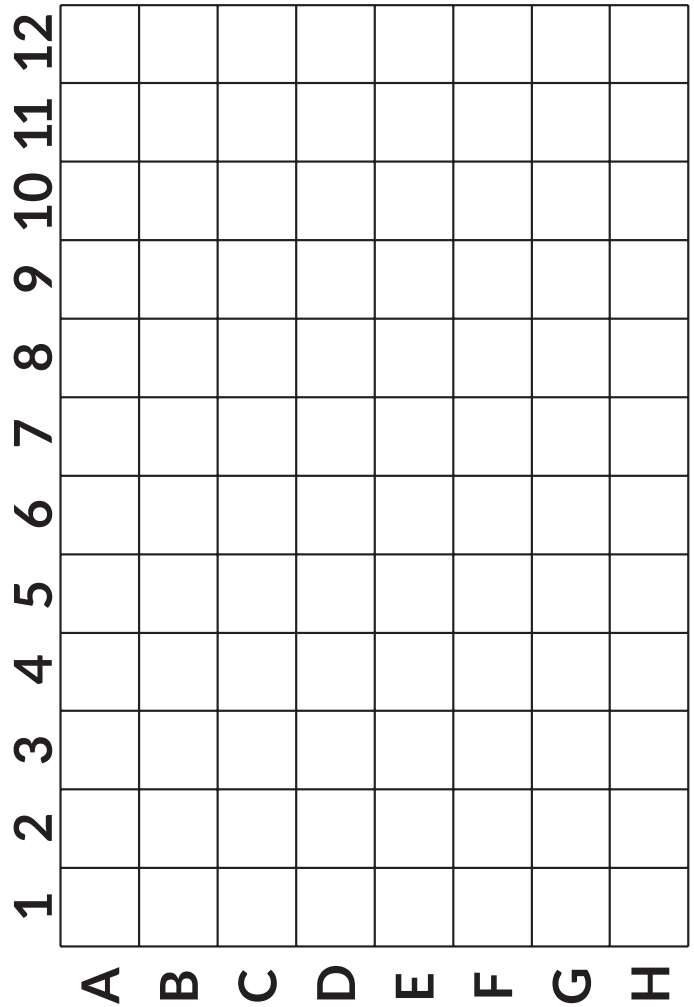
RESOURCES

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 wells	Sample was too dilute	Re-assay the sample using less of a dilution
Sample absorbance values are above the highest point in standard curve	Creatinine concentration was too high in the sample	Dilute samples with pure water and re-assay.
The creatinine standard curve did not work	Either the creatinine standards were not diluted properly or the creatinine standard has deteriorated	Set up the standards according to Table 2 (on page 14) and re-assay

References

1. Wyss, M. and Kaddurah-Daouk, R. Creatine and creatinine metabolism. *Physiol. Rev.* **80(3)**, 1107-1213 (2000).
2. Kashani, K., Rosner, M.H., and Ostermann, M. Creatinine: From physiology to clinical application. *Eur. J. Intern. Med.* **72**, 9-14 (2020).
3. Bowers, L.D. and Wong, E.T. Kinetic serum creatinine assays. II. A critical evaluation and review, *Clin. Chem.* **26(5)**, 555-561 (1980).
4. Slot, C. Plasma creatinine determination. A new and specific Jaffe reaction method. *Scand. J. Clin. Lab. Invest.* **17(4)**, 381-387 (1965).
5. Heinegård, D. and Tiderström, G. Determination of serum creatinine by a direct colorimetric method. *Clin. Chim. Acta* **43(3)**, 305-310 (1973).
6. Cook, J.G.H. Factors influencing the assay of creatinine. *Ann. Clin. Biochem.* **12(6)**, 219-232 (1975).



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.