Creatinine (urinary) Colorimetric Assay Kit

Item No. 500701

Customer Service 800.364.9897 * **Technical Support** 888.526.5351
www.caymanchem.com

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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 laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

Precautions

Please read these instructions carefully before beginning this assay.

For research use only. Not for human or diagnostic use.

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

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

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. Adjustable pipettes and a repeating pipettor
3. A source of pure water; glass distilled water or HPLC-grade water is acceptable

INTRODUCTION

Background

Creatine synthesized in kidney, liver, and pancreas is transported in blood to muscle and brain where it is phosphorylated to phosphocreatine. Some free creatine in muscle is converted to creatinine. The amount of creatinine produced is proportional to the individuals muscle mass. In the absence of renal disease, the excretion rate of creatinine in an individual is relatively constant. Thus, urinary creatinine levels may be used as an index of standardization for other tests. Measurement of creatinine clearance is also useful in detecting renal disease and estimating the extent of impairment of renal function.[1]

About This Assay

Cayman's Creatinine (urinary) Colorimetric Assay 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.[2] 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.[1,3-4] The sample creatinine concentration is determined using a creatinine standard curve.

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 (Item No. 10008477), 6 ml of Creatinine Surfactant (Item No. 10008478), 10 ml of Creatinine Color Reagent (Item No. 10005315), and 3.6 ml of Creatinine NaOH (Item No. 10005316)). 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 HPLC-grade 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.

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 1, below. We suggest you record the contents of each well on the template sheet provided (see page 19).

	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 1. 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 1, below. Take eight clean glass test tubes and label them A-H. Add the amount of Creatinine Standard and HPLC-grade water to each tube as described in Table 1, below.

Tube	Creatinine Standard (µl)	HPLC-grade 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 1. Concentration of Standards

Performing the Assay

1. **Creatinine Standard Wells** - Add 15 µl of Creatinine Standard (tubes A-H) per well in the designed wells on the plate (see suggested plate configuration, Figure 1, page 9).
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 HPLC-grade 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 (I_{abs}).
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 (F_{abs}).

ANALYSIS

Calculations

1. Calculate the average Initial absorbance (I_{abs}) of each standard and sample.
2. Calculate the average Final absorbance (F_{abs}) 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 1 (on page 11). See Figure 2, on page 15, 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.

$$\textbf{Creatinine (mg/dl)} = \left[\frac{\text{sample absorbance - (y-intercept)}}{\text{slope}}\right] \text{ x sample dilution}$$

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).

Assay Range:

Under the standardized conditions of the assay described in this booklet, the dynamic range of the kit is 0-15 mg/dl of creatinine.

Representative Standard Curve

The standard curve presented on page 15 is an example of the data typically provided 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.

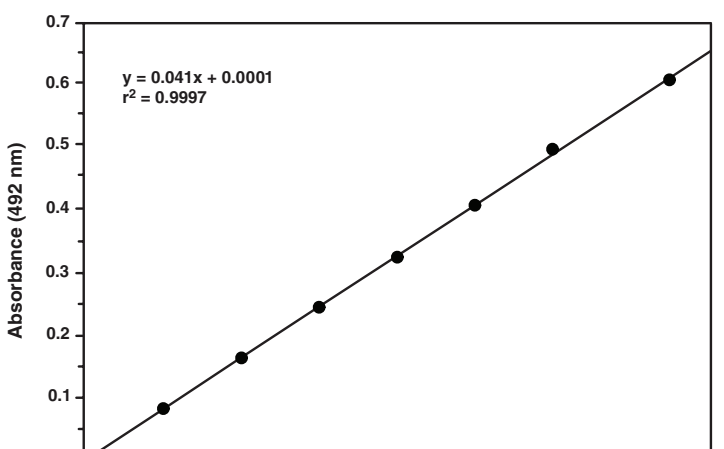


Figure 2. Creatinine standard curve

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 highest point in standard curve	Creatinine concentration was too high in the sample	Dilute samples with HPLC-grade 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 1 (on page 11) 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. Heinegård, D. and Tiderström, G. Determination of serum creatinine by a direct colorimetric method. *Clinica. Chimica. Acta.* **43**, 305-310 (1973).
4. Cook, J.G.H. Factors influencing the assay of creatinine. *Ann. Clin. Biochem.* **12**, 219-232 (1975).

Related Products

p-Aminohippuric Acid (PAH) Assay Kit - Item No. 700880
Antioxidant Assay Kit - Item No. 709001
Arginine Vasopressin EIA Kit - Item No. 583951
Chloride Colorimetric Assay Kit - Item No. 700610
Cortisol Express EIA Kit - Item No. 500370
Creatine Kinase Fluorometric Assay Kit - Item No. 700630
Creatinine (serum) Colorimetric Assay Kit - Item No. 700460
Cyclic AMP EIA Kit - Item No. 581001
Cyclic GMP EIA Kit - Item No. 581021
Cysteinyl Leukotriene EIA Kit - Item No. 500390
Hemoglobin Colorimetric Assay Kit - Item No. 700540
β-Hydroxybutyrate (Ketone Body) Fluorometric Assay Kit - Item No. 700740
Inulin Fluorometric Assay Kit - Item No. 700770
8-Isoprostane EIA Kit - Item No. 516351
D-Lactate Assay Kit - Item No. 700520
L-Lactate Assay Kit - Item No. 700510
Leukotriene E_4 EIA Kit - Item No. 520411
Malate Fluorometric Assay Kit - Item No. 700790
Nitrate/Nitrite Colorimetric Assay Kit - Item No. 780001
Nitrate/Nitrite Colorimetric Assay Kit (LDH method) - Item No. 760871
Oxytocin EIA Kit - Item No. 500440
Protein Determination Kit - Item No. 704002
Pyruvate Assay Kit - Item No. 700470
Thromboxane B_2 Express EIA Kit - Monoclonal - Item No. 10004023
Urea Fluorometric Assay Kit - Item No. 700620
Uric Acid Assay Kit - Item No. 700320

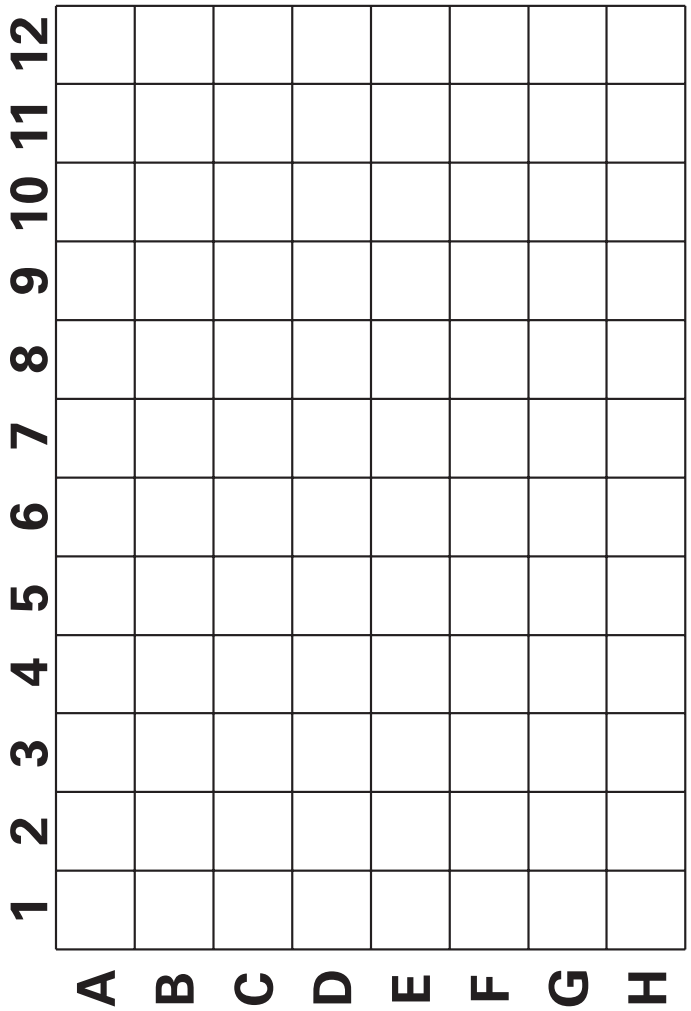
Warranty and Limitation of Remedy

Cayman Chemical Company makes no warranty or guarantee of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman warrants only to the original customer that the material **will meet our specifications at the time of delivery**. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have any obligation or liability, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer's exclusive remedy and Cayman's sole liability hereunder shall be limited to a **refund** of the purchase price, or at Cayman's option, the **replacement**, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.



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