ab204537

Creatinine Assay Kit (Colorimetric)

Instructions for Use

For the quantitative determination of Creatinine in urine samples.

This product is for research use only and is not intended for diagnostic use.

Version 3a Last Updated 20 December 2018

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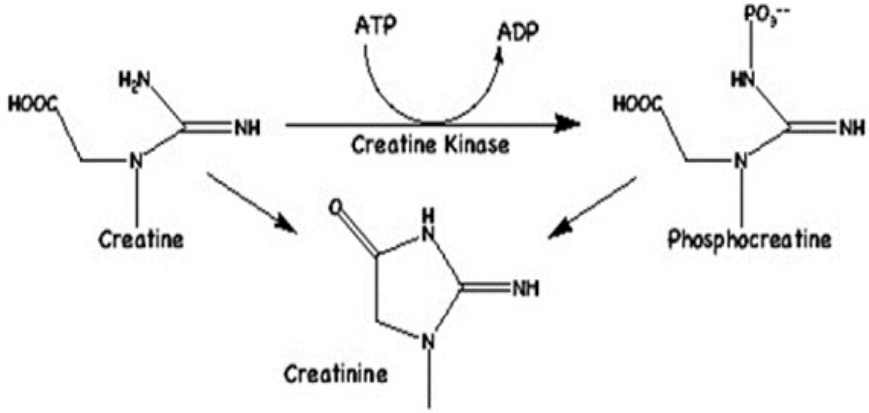
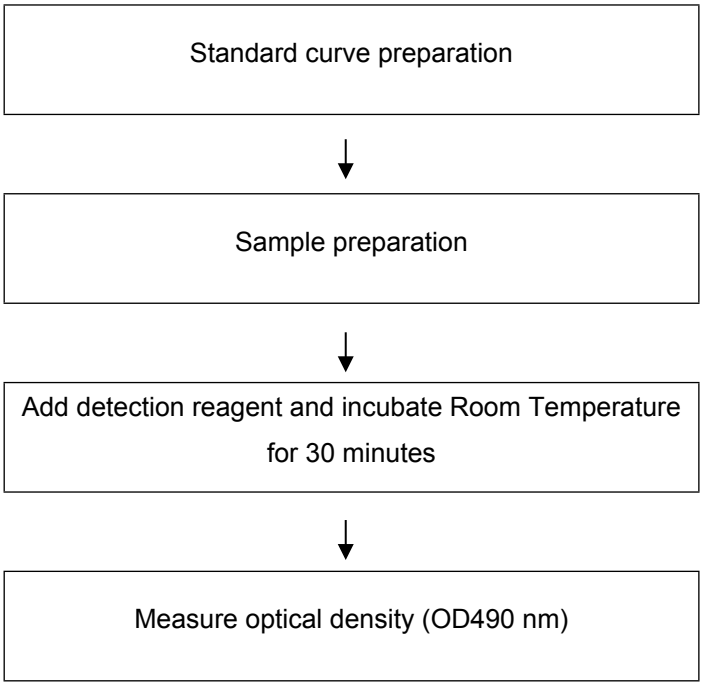
1. BACKGROUND

Creatinine Assay Kit (Colorimetric) (ab204537) is a complete kit for the quantitative determination of Creatinine in urine, and is based on the Jaffe reaction.

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. Creatine either comes from the diet or is 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. 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. Creatinine forms spontaneously from p-creatine, and under normal conditions, its formation occurs at a relatively constant rate. Intra-individual variation of creatinine levels is <15% from day to day, making it a useful marker for normalizing levels of other molecules found in urine. Altered creatinine levels may be associated with conditions that result in decreased renal blood flow, such as diabetes and cardiovascular disease.



2. ASSAY SUMMARY



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. Modifications to the kit components or procedures may result in loss of performance.

Creatinine Detection Reagent contain picric acid. Avoid contact of solution with skin and eyes. Picric acid is an irritant and if dried, potentially explosive. Avoid contact with metals and use large volumes of water during disposal.

4. STORAGE AND STABILITY

Store kit at 4ºC 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 section 5.

Aliquot components in working volumes before storing at the recommended temperature.

5. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- 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

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Clear Microtiter Plates	2 units	+2-8ºC	+2-8ºC
Creatinine Standard (100 mg/dL)	1 vial	+2-8ºC	+2-8ºC
Creatinine Detection Reagent	20 mL	+2-8ºC	+2-8ºC
Plate Sealers	2 units	+2-8ºC	+2-8ºC

7. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- MilliQ water or other type of double distilled water (ddH_2O).
- Microcentrifuge tubes.
- Pipettes and pipette tips.
- Colorimetric microplate reader – equipped with filter for OD= 490 nm.

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.**
- Keep enzymes, heat labile components and samples on ice during the assay.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- 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.
- Pipet the reagents to the side of the wells to avoid possible contamination.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **Creatinine Standard:**

Ready to use as supplied. Aliquot standard so that you have enough volume to perform the desired number of assays. Store at +2-8°C. Keep on ice while in use.

9.2 **Creatinine Detection Reagent:**

Ready to use as supplied. Store at +2-8°C. Keep on ice while in use.

9.3 **Clear microtiter plates:**

Ready to use as supplied. Store at +2-8°C.

9.4 **Plate sealers:**

Ready to use as supplied. Store at +2-8°C.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 2 hours.

10.1 Prepare a 20 mg/dL standard by diluting 200 µL of the provided 100 mg/dL standard with 800 µL ddH_2O.

10.2 Using the 20 mg/dL standard, prepare standard curve dilutions as described in the table in plastic or glass tubes:

Standard #	Sample to Dilute	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (mg/dL)	Final Conc. (mg/dL)
1	Stock	200	800	100	20
2	Standard #1	500	500	20	10
3	Standard #2	500	500	10	5
4	Standard #3	500	500	5	2.5
5	Standard #4	500	500	2.5	1.25
6	Standard #5	500	500	1.25	0.625
7	Standard #6	500	500	0.625	0.3126

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

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. Alternatively, if that is not possible, we suggest that you snap freeze the samples in liquid nitrogen upon extraction and store them 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.
- This assay has been validated for use with Human, mouse, rat, dog and monkey urine samples.
- Twenty random clean catch Human urine samples were tested in the assay. Values ranged from 3 to 182 mg/dL with an average of 64 mg/dL.

11.1 Urine samples:

Urine samples can be tested directly by adding sample to the microplate wells. Samples containing visible protein or particulates should be centrifuged or filtered prior to using.

However, to find the optimal values and ensure your readings will fall within the standard values, we recommend performing several dilutions of the sample. A minimum 1:20 dilution of urine samples into ddH_2O is recommended to remove matrix interference and obtain accurate results.

Rhesus monkey urine samples should be diluted 1:2 due to low levels of creatinine.

NOTE: *We suggest using different volumes of sample to ensure readings are within the Standard Curve range*

12. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate**

12.1 **Set up Reaction wells:**

- Standard wells = 50 µL standard dilutions.
- Sample wells = 50 µL diluted samples
- Blank wells= 50 µL ddH_2O

12.2 Add 100 µL of Creatinine Detection Reagent to each well.

12.3 Mix well by gently tapping the sides of the plate.

12.4 Incubate for 30 minutes at room temperature.

12.5 Blank the plate reader against the water blank.

12.6 Measure output on a microplate reader at OD=490 nm. If plate reader is not capable of adjusting for the blank, manually subtract the mean OD of the substrate blank from all readings.

13. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 Subtract the mean absorbance value of the blank (50 µL ddH_2O on Step 12.1) from all standard and sample readings. This is the corrected absorbance.

13.3 Plot the corrected absorbance values for each standard as a function of the final concentration of creatinine.

13.4 Draw the best smooth curve through these points to construct a standard curve. Most plate reader software or graphing software can plot these values and curve fit. A four parameter algorithm (4PL) usually provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic).

13.5 Interpolate creatinine concentrations for unknown samples from the standard curve plotted.

13.6 Concentration of Creatinine (mg/dL) in the test samples is calculated as:

$$Creatinine = A * D$$

Where:

A = Amount of Creatinine in the sample well (mg/dL).

D = Sample dilution factor.

14. TYPICAL DATA

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

The following data are obtained using the different concentrations of standard as described in this protocol:

Sample	Mean OD	Net OD	Creatinine mg/dL
Blank	0.137	---	0
S1	2.203	2.066	20
S2	1.206	1.069	10
S3	0.680	0.543	5
S4	0.411	0.274	2.5
S5	0.278	0.141	1.25
S6	0.207	0.070	0.625
S7	0.172	0.035	0.3125

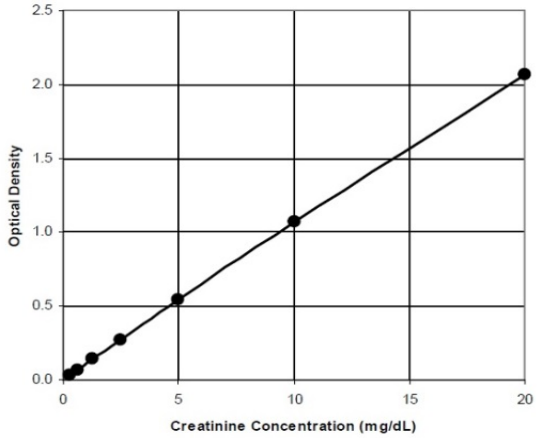


Figure 1: Typical creatinine standard calibration curve using colorimetric reading. The sensitivity or limit of detection was measured by determining mean signal at background (0 pg/mL) for 20 replicates and calculating corresponding concentration using data from 10 standard curves. The sensitivity was found to be 0.042 ng/dL.

INTRA-ASSAY PRECISION

Determined by assaying 20 replicates of four Human urine samples diluted 1:20 with deionized water.

mg/dL	% CV
7.981	2.159
7.452	3.203
2.007	5.995
7.326	3.729

INTER-ASSAY PRECISION

Determined by measuring duplicate concentration values of four Human urine samples diluted 1:20 in deionized water in 20 assays run over the course of several days.

mg/dL	% CV
7.591	2.249
7.232	2.052
1.932	2.027
7.085	1.433

SPIKE AND RECOVERY

Creatinine was spiked to three different levels in 10 urine samples diluted 1:20 in dH_2O and compared to the same spike levels in dH_2O. The recovery for the high, medium and low spike was 93%, 92% and 99% respectively. The mean recovery was 95%.

Creatinine spike mg/dL	Mean % Recovery	Mean % Recovery
8.0	93	95
4.0	92	
4.0	99	

LINEARITY

Twenty urine samples were serially diluted 1:2 in ddH_2O and run in the assay. Dilutional linearity was calculated for each sample based on the observed values at the highest dilution that read above the limit of detection (LOD). Mean dilutional linearity was averaged across the twenty samples. The results are shown in the table below.

Dilution	% dilutional linearity urine
Neat	67
1:2	101
1:4	95
1:16	93
1:32	96
1:64	101
1:128	103
1:256	100

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.*

- Prepare standard, (aliquot if necessary); get equipment ready.
- Prepare standard curve.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up plate for standard (50 µL), samples (50 µL) and blank wells (50 µL).
- Add 100 µL of Creatinine Detection Reagent to each well.
- Gently mix by tapping plate.
- Incubate plate at RT 30 min.
- Measure plate at OD = 490 nm.

16. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Samples give higher value than the highest standard	Starting sample concentration is too high.	Dilute the specimens and repeat the assay
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the kit	Store the all components as directed.

17. **FAQ**

18. INTERFERENCES

These chemicals or biological materials will cause interferences in this assay causing compromised results or complete failure:

- Bilirubin, glucose - typical components of Human urine may interfere with the Jaffe reaction for urinary creatinine measurement.

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

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 400 921 0189 / +86 21 2070 0500

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp