

Creatinine Assay Kit

Catalog # 6041

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	Assay kit to quantify creatinine
FORMAT:	96-well ELISA Plate with removeable strips
ASSAY TYPE:	Colorimetric
ASSAY TIME:	35 minutes
STANDARD RANGE:	400 µg/ml to 6.3 µg/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) samples/plate
SAMPLE TYPES:	Urine, Serum, and Plasma
RECOMMENDED SAMPLE DILUTIONS:	Varies
CHROMOGEN:	N/A (read at 492 nm)
STORAGE:	Room Temperature
VALIDATION DATA:	Intra-Assay (1.8-4.1%)/Inter-Assay (2.8-7.8%)/Spiking Test (100-108%)
NOTES:	

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INTRODUCTION

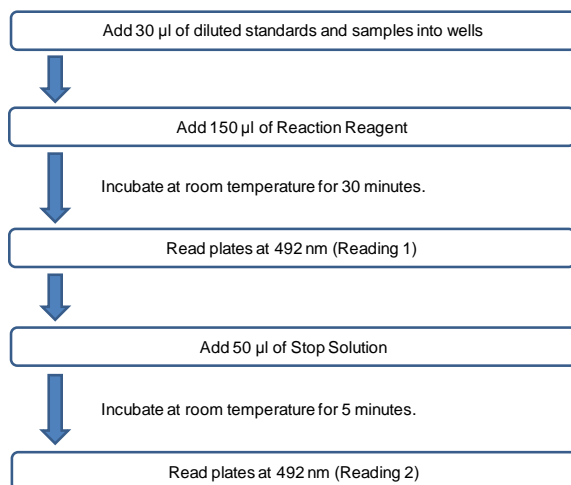
Creatinine (2-Amino-1-methyl-2-imidazolin-4-one) is a product of creatine kinase activity in skeletal muscle. Therefore serum creatinine levels are consistent depending on an individual's muscle amount (1). Serum creatinine is absorbed by the kidneys via glomerular filtration and then excreted. Determining the glomerular filtration rate (GFR) using creatinine levels is a useful tool to evaluate renal function in renal diseases and impairments (2-4). In addition, urinary creatinine levels are commonly used as an index of standardization for a variety of other tests (5-7).

Chondrex, Inc provides a Creatinine Assay Kit (Cat # 6041) employing Jaffe Reaction (8). The assay only requires 30 μ l of samples and a 30-minute assay time using a standard range of 400 - 6.3 μ g/ml. To standardize assay results between samples from human patients and animal models, this kit can be used together with Urinary Protein Assays (Cat # 6026 and 9040), Albumin Detection ELISA Kits (Cat # 3012 and 3020), the NTX-I Detection ELISA Kit (Cat # 6040), and the CTX-I Detection ELISA Kit (Cat # 6033). Please contact support@chondrex.com or visit www.chondrex.com for more information.

KIT COMPONENTS

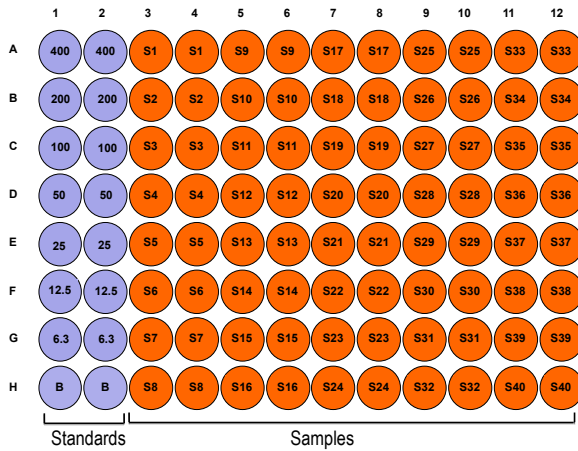
Item	Quantity	Amount	Storage
Creatinine Standard (60411)	1 vial	400 μ g, lyophilized	RT
Solution A (60414)	1 Bottle	5 ml	RT
Solution B (60415)	1 Bottle	20 ml	RT
PBS (60264)	1 Bottle	50 ml	RT/4°C
Stop Solution (9016)	1 Bottle	10 ml	RT/-20°C
96-Well ELISA Plate	1 each	8-well Strips x12	RT

ASSAY OUTLINE



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PLATE MAPPING



NOTES BEFORE USING ASSAY

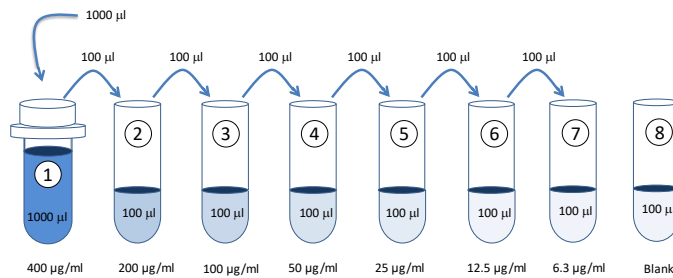
NOTE 1: It is recommended that the standard and samples be run in duplicate.

NOTE 2: Warm up all buffers to room temperature before use.

NOTE 3: This kit contains animal components from non-infectious animals and should be treated as potential biohazards in use and for disposal.

ASSAY PROCEDURE

- Prepare Standard Dilutions:** The recommended standard range is 6.3 - 400 µg/ml. Dissolve one vial of creatinine standard in 1 ml of PBS (60264) for the 400 µg/ml standard. Then serially dilute 400 µg/ml of creatinine standard solution with Solution A. For example, mix 100 µl of the standard (400 µg/ml) with an equal volume of Solution A in a 0.5 ml microcentrifuge tube to make a 200 µg/ml solution, and then repeat it five more times for 100, 50, 25, 12.5, and 6.3 µg/ml solutions. The remaining 400 µg/ml standard stock can be stored at 4°C for use in a second assay. Chondrex, Inc. recommends making fresh serial dilutions for each assay.



- Prepare Sample Dilutions:** Centrifuge samples at 10,000 rpm at 4°C for 3 minutes to remove insoluble materials and lipids. Dilute the supernatants with Solution A if it is necessary. Two to three different sample dilutions are recommended if the creatinine levels in the samples are unknown.
- Add Standards and Samples:** Add 30 µl of Solution A (blank), standards, and samples to designated wells.

4. **Prepare Reaction Reagent:** Mix Solution A and Solution B 1:4 in a glass tube. For example, one well requires 40 μ l of Solution A mixed with 120 μ l of Solution B. The following shows a protocol for preparing reaction reagent by the number of strips in an assay (including extra volume).

Strip #	Solution A (ml)	Solution B (ml)
2	0.8	3.2
4	1.5	6.0
6	2.0	8.0
8	2.5	10.0
10	3.0	12.0
12	4.0	16.0

5. **Add Reaction Reagent:** Add 150 μ l of reaction reagent to each well and incubate at room temperature for 30 minutes.
6. **Read Plate:** Read the OD values at 492 nm (Reading 1).
7. **Add Stop Solution:** Add 50 μ l of Stop Solution to each well and incubate at room temperature for 5 minutes.
8. **Read Plate:** Read the OD values at 492 nm (Reading 2).

CALCULATING RESULTS

- Calculate the average of the duplicate OD values of Reading 1 for the blank, standards, and test samples.
- Subtract the “blank” (B) values from the averaged OD values in step 1 (Corrected Reading 1).
- Calculate the average of the duplicate OD values of Reading 2 for the blank, standards, and test samples.
- Subtract the “blank” (B) values from the averaged OD values in step 3 (Corrected Reading 2).
- Subtract the Corrected Reading 2 from Corrected Reading 1 in corresponding wells. These are the Corrected OD values. This step eliminates background noise OD values caused by the samples themselves.
- Plot the Corrected OD values of standards against the concentration of creatinine (μ g/ml). Using a log/log plot will linearize the data. Figure 1 shows a representative experiment where the standard range is 6.3 - 400 (μ g/ml)
- The μ g/ml of creatinine in samples can be calculated using regression analysis on the Corrected OD sample values.

Figure 1 - A Typical Standard Curve for the Creatinine Assay Kit

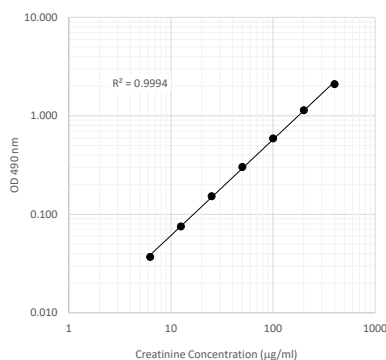


Table 1 - Reproducibility Data for the Creatinine Assay Kit

Test	12.5 µg/ml	50 µg/ml	200 µg/ml
Intra-Assay CV (%)	2.9	1.8	4.1
Inter-Assay CV (%)	7.8	3.2	2.8
Spike Test* (%)	108%	101%	100%

* Known amounts of creatinine were added to samples and then diluted with Solution A.

TROUBLESHOOTING

For frequently asked questions about assays and ELISAs, please see Chondrex, Inc.'s [Assay FAQ](#) for more information.

REFERENCES

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