# 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: | Serum and plasma samples require pretreatment for deproteinization. |

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## INTRODUCTION

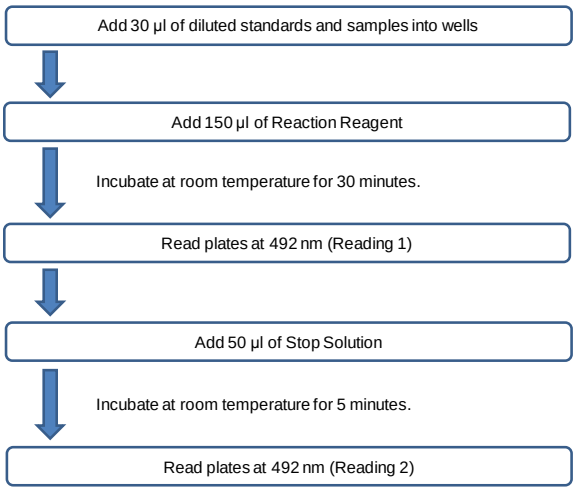
Creatinine (2-Amino-1-methyl-2-imidazolin-4-one) is a product of creatine kinase activity in skeletal muscle. Therefore serum creatinine levels are consistent depending on an individual's muscle amount (1). Serum creatinine is absorbed by the kidneys via glomerular filtration and then excreted. Determining 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 the Jaffe Reaction (8). The assay only requires 30 µl of samples and a 30-minute assay time using a standard range of 400 - 6.3 µg/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 µg, lyophilized | RT |
| Solution A (60414) | 1 Bottle | 5 ml | RT |
| Solution B (60415) | 1 Bottle | 20 ml | RT |
| 1X 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

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 400 | 400 | S1 | S1 | S9 | S9 | S17 | S17 | S25 | S25 | S33 | S33 |
| B | 200 | 200 | S2 | S2 | S10 | S10 | S18 | S18 | S26 | S26 | S34 | S34 |
| C | 100 | 100 | S3 | S3 | S11 | S11 | S19 | S19 | S27 | S27 | S35 | S35 |
| D | 50 | 50 | S4 | S4 | S12 | S12 | S20 | S20 | S28 | S28 | S36 | S36 |
| E | 25 | 25 | S5 | S5 | S13 | S13 | S21 | S21 | S29 | S29 | S37 | S37 |
| F | 12.5 | 12.5 | S6 | S6 | S14 | S14 | S22 | S22 | S30 | S30 | S38 | S38 |
| G | 6.3 | 6.3 | S7 | S7 | S15 | S15 | S23 | S23 | S31 | S31 | S39 | S39 |
| H | B | B | S8 | S8 | S16 | S16 | S24 | S24 | S32 | S32 | S40 | S40 |

Standards | Samples

## NOTES BEFORE USING ASSAY

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

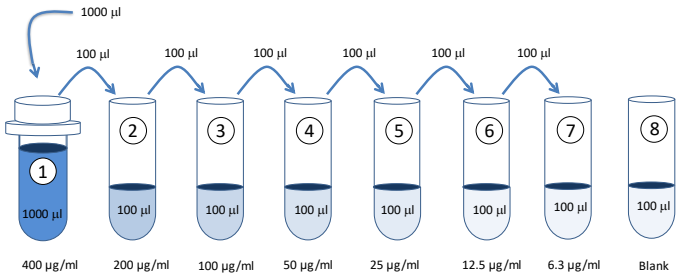
NOTE 2: Warm up all buffers to room temperature before use.

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

NOTE 4: Proteins in the samples, especially bilirubin, may interfere with the assay. Serum and plasma samples require a deproteinization pretreatment step before running this assay. Please see the following sample pretreatment protocol. Mix each plasma or serum sample with 9% trichloroacetic acid (not provided) at a ratio of 2:1 (sample volume: reagent volume) and incubate for 10 minutes. Centrifuge samples at 1200 x g for 10 minutes and collect the supernatants. Use the supernatants as the samples in this creatinine assay. The assay results for these samples must be multiplied by 1.5 to account for the dilution factor.

## ASSAY PROCEDURE

1. **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 PBS. For example, mix 100 µl of the standard (400 µg/ml) with an equal volume of PBS 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.

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2. **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 PBS if it is necessary. Two to three different sample dilutions are recommended if the creatinine levels in the samples are unknown.

   NOTE: serum and plasma samples require a pretreatment process. Please see the "Notes Before Using Assay" section above for more information.

3. **Add Standards and Samples**: Add 30 µl of PBS (blank), standards, and samples to designated wells.

4. **Prepare Reaction Reagent**: Mix Solution A and Solution B at a ratio of 1:4 in a glass tube. For example, one well requires 40 µl of Solution A mixed with 120 µl 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 µl 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 µl 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

1. Calculate the average of the duplicate OD values of Reading 1 for the blank, standards, and test samples.

2. Subtract the "blank" (B) values from the averaged OD values in step 1 (Corrected Reading 1).

3. Calculate the average of the duplicate OD values of Reading 2 for the blank, standards, and test samples.

4. Subtract the "blank" (B) values from the averaged OD values in step 3 (Corrected Reading 2).

5. 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.

6. Plot the Corrected OD values of standards against the concentration of creatinine (µg/ml). Using a log/log plot will linearize the data. Figure 1 shows a representative experiment where the standard range is 6.3 - 400 (µg/ml)

7. The µg/ml of creatinine in samples can be calculated using regression analysis on the Corrected OD sample values.

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

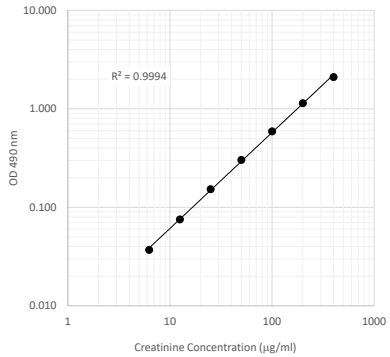


Table 1 - Reproducibility Data for the Creatinine Assay Kit

| 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 PBS.

## TROUBLESHOOTING

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

## REFERENCES

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