Product Manual

Creatinine Colorimetric Detection Kit

Catalog #: ADI-907-030A

2x96 well Colorimetric Detection Kit
For use with urine samples

For the latest product information, including support documentation, visit us online:
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Product Manual

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Please read entire booklet before proceeding with the assay.

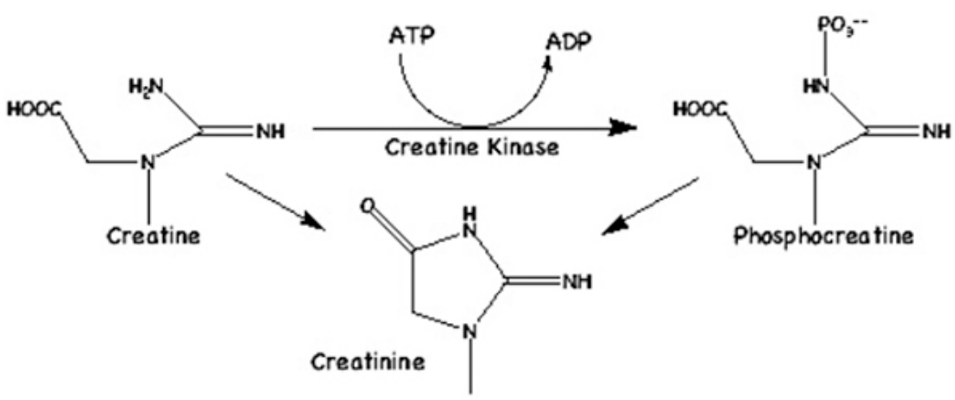
Carefully note the handling and storage conditions of each kit component.

Please contact Enzo Life Sciences Technical Support if necessary.

INTRODUCTION

The Creatinine Detection Kit is a complete kit for the quantitative determination of creatinine in urine, and is based upon the Jaffe reaction. Please read the complete kit insert before performing this assay.

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[1]. 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[2]. 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 pH2. Creatinine forms spontaneously from p-creatine[3], 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[4-6].



PRINCIPLE

1. Samples and standards are added to uncoated clear microtiter plate wells
2. Creatinine Detection Reagent is added to the wells. The plate is incubated.
3. The resulting color, which is directly proportional to the concentration of creatinine, is read at 490nm.

Do not mix components from different kit lots or use reagents beyond the expiration date of the kit.

The standard should be handled with care due to the known and unknown effects of the antigen.

Creatinine Detection Reagent contains picric acid. The solution should not come in contact with skin or eyes. Picric acid is an irritant and, if dried, potentially explosive. Avoid contact with metals and use large volumes of water during disposal.

MATERIALS SUPPLIED

1. **Clear Microtiter Plates, Catalog No. 80-2394:**

   Two plates of 96 wells, Clear uncoated microtiter plates.

2. **Creatinine Standard, 100 mg/dl, Catalog No. 80-2395:**

   Creatinine solution in deionized water; calibrated to a NIST creatinine standard.

3. **Creatinine Detection Reagent, 20 mL Catalog No. 80-2396:**

   A specially formulated single-step solution to detect creatinine

4. **Plate Sealers, 2 each, Catalog No. 30-0012:**

STORAGE

All components of this kit should be stored at 4°C until the kit's expiration date.

OTHER MATERIALS NEEDED

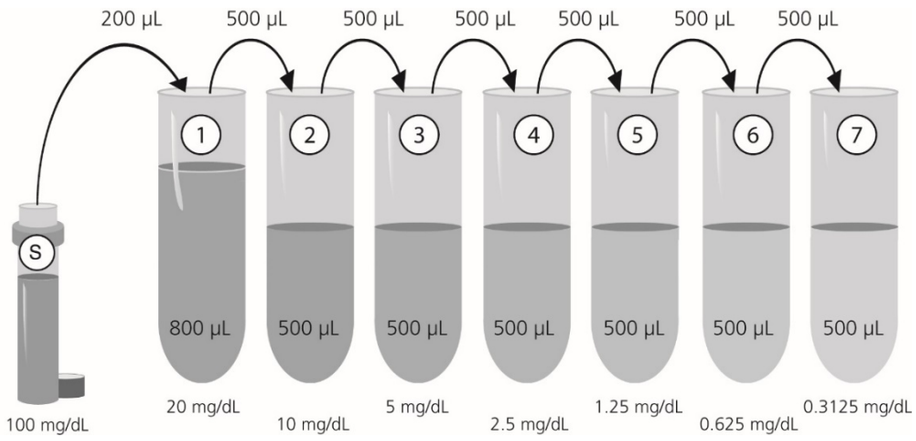
1. Distilled or deionized water
2. Colorimetric 96 well microplate reader capable of reading optical density at 490nm, preferably with correction at between 570 and 590nm.
3. Software for converting optical density readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting.
4. Precision pipets for volumes between 5 µL and 1,000 µL.
5. Repeater pipet for dispensing 100 µL.

REAGENT PREPARATION

Bring all reagents to room temperature for at least 30 minutes prior to opening.

Creatinine Standard Curve

Label seven glass test tubes #1 through #7. Pipet 800 µL of distilled or deionized water into tube #1 and 500 µL into tubes #2-#7. Carefully add 200 µL of the Creatinine Standard stock solution to tube #1 and vortex completely. Take 500 µL of the creatinine solution in tube #1 and add it to tube #2 and vortex completely. Add 500 µL of tube #2 to tube #3 and vortex completely. Repeat this for tubes #4 through #7.



Diluted standards should be used within 2 hours of preparation. The concentration of creatinine in tubes is labeled above. Refer to the plate layout at the end of this document to determine the number of wells to be used.

SAMPLE HANDLING

This assay has been validated for use with human, mouse, rat, dog and monkey urine samples. Urine samples containing visible protein or particulates should be centrifuged or filtered prior to using. Rhesus monkey urine samples should be diluted 1:2 due to low levels of Creatinine. A minimum 1:20 dilution of urine samples into distilled or deionized water is recommended to remove matrix interference and obtain accurate results in the assay. Samples with concentrations outside the standard curve range will need to be reanalyzed using a different dilution. Diluted samples should be used within 2 hours of preparation.

Twenty random clean catch human urine samples were tested in the assay. Values ranged from 3 to 182 mg/dl with an average of 64mg/dl.

The Jaffe reaction used in this assay has been modified to read creatinine levels in urine[7,8]. Some typical components of human urine may interfere with the Jaffe reaction for urinary creatinine measurement[9,10].

ASSAY PROCEDURE

Bring all reagents to room temperature for at least 30 minutes prior to opening.

Refer to the Plate Layout to determine the number of wells to be used.

1. Pipet 50 µL of diluted samples, standards, and blank (distilled or deionized water) to the bottom of the appropriate wells.
2. Add 100 µL of the Creatinine Detection Reagent to each well using a repeater or multichannel pipet.
3. Gently tap the sides of the plate to ensure adequate mixing of the reagents.
4. Seal the plate and incubate at room temperature for 30 minutes.
5. Remove seal and zero the plate reader against the water blank before reading the optical density at 490nm. If plate reader is not capable of adjusting for the blank, manually subtract the mean OD of the substrate blank from all readings.

All standards and samples should be run in duplicate.

Pre-rinse each pipet tip with reagent. Use fresh pipet tips for each sample, standard, and reagent.

Pipet the reagents to the sides of the wells to avoid possible contamination.

CALCULATION OF RESULTS

Several options are available for the calculation of the concentrations of Creatinine in samples. We recommend that the data be handled by an immunoassay software package utilizing a 4-parameter logistic curve fitting program. Such software is often supplied by most plate reader manufacturers. The 4-parameter logistic curve fit provides more accurately interpolated sample values compared to a linear curve fit. Assay Blaster! assay analysis software (Cat. #ADI-28-0002) is an easy-to-use and cost effective program that provides the options of point-to-point, 4PL and 5PL curve fit-ting options.

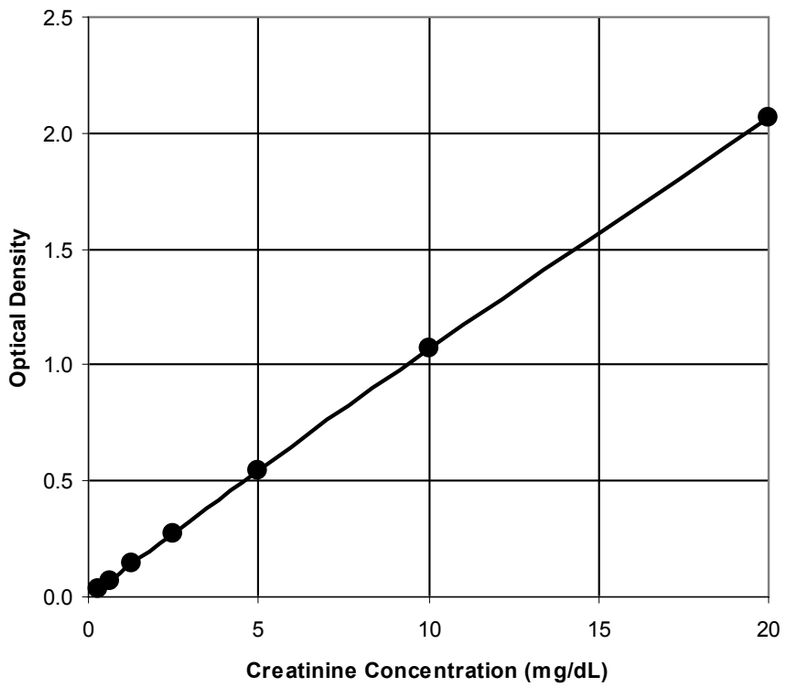
Samples with concentrations outside the standard curve range will need to be reanalyzed using a different dilution.

TYPICAL RESULTS

The results shown below are for illustration only and should not be used to calculate the results from another assay.

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**

TYPICAL STANDARD CURVES



PERFORMANCE CHARACTERISTICS

Sensitivity

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.

Sensitivity:
0.042 mg/dl

Linearity

Twenty urine samples were serially diluted 1:2 in diH_2O and run in the assay. Dilution-al 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.

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

Spike & Recovery

Creatinine was spiked to three different levels in ten urine samples diluted 1:20 in diH_2O and compared to the same spike levels in diH_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	
1.0	99	

Precision

Intra-assay precision was determined by assaying 20 replicates of four human urine samples diluted 1:20 with deionized water.

Intra-assay precision	
(mg/dl)	%CV
7.981	2.159
7.452	3.203
2.007	5.995
7.326	3.729

Inter-assay precision was 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.

Inter-assay precision	
(mg/dl)	%CV
7.591	2.249
7.232	2.052
1.932	2.027
7.085	1.433

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10) Young, D.D., in “Effects of Drugs on Clinical laboratory Tests”, 1990.

PLATE LAYOUT

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLANK	BLANK										
B	S1: 20 mg/dl	S1: 20 mg/dl										
C	S2: 10 mg/dl	S2: 10 mg/dl										
D	S3: 5 mg/dl	S3: 5 mg/dl										
E	S4: 2.5 mg/dl	S4: 2.5 mg/dl										
F	S5: 1.25 mg/dl	S5: 1.25 mg/dl										
G	S6: 0.625 mg/dl	S6: 0.625 mg/dl										
H	S7: 0.325 mg/dl	S7: 0.325 mg/dl										

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