Creatinine Urinary Detection Kit

Catalog Number EIACUN (192 tests)

Rev 1.0

For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Creatinine Urinary Detection Kit is designed to detect and quantify the level of creatinine in urine samples. The assay was validated with samples from human, rat, dog, and monkey, but is expected to measure creatinine in samples from most other species. Mouse urine samples are not compatible with the assay as renal secretion rather than filtration accounts for over half of murine urinary creatinine.

Creatinine (2-amino-1-methyl-5H-imadazol-4-one) is a metabolite of creatine and phosphocreatine (p-creatine). It is converted through a non-enzymatic process, diffuses into the blood, and is excreted by the kidneys. The conversion appears to be irreversible *in vivo*, and is favored by higher temperatures and lower pH *in vitro*.

Creatinine forms spontaneously from p-creatine at a rate that is relatively constant and with an intra-individual variation of <15% from day to day, making it a useful tool for normalizing the levels of other molecules found in urine.

Contents and storage

Kit and components are shipped at –20°C. Upon receipt, store the kit at –20°C. Once open, store the kit at 4°C and use within 2 weeks.

Components	Quantity
Creatinine Standard; 100 mg/dL creatinine in deionized water	1 mL
Clear 96-well Plate	2 plates
Creatinine Reagent; contains picric acid, irritant	20 mL
Plate sealer	2

Materials required but not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 490 nm
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution

Procedural guidelines

Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Dilute samples

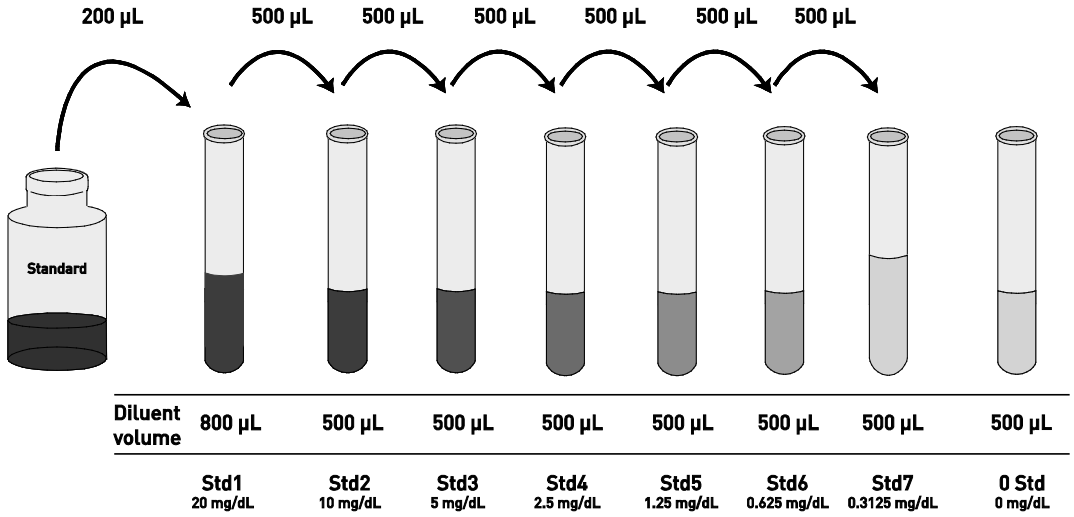
Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

- Dilute **rhesus monkey urine** samples 1:2 in distilled or deionized water.
- Dilute **all other urine** samples ≥1:20 in distilled or deionized water.
- Use all samples within **2 hours** of dilution.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

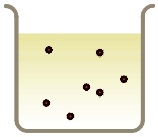
1. Add 200 µL Creatinine Standard to one tube containing 800 µL distilled or deionized water and label as 20 mg/dL creatinine.
2. Add 500 µL distilled or deionized to each of 7 tubes labeled as follows: 10, 5, 2.5, 1.25, 0.625, 0.3125, and 0 mg/dL creatinine.
3. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
4. **Use the standards within 2 hours of preparation**.



Assay procedure

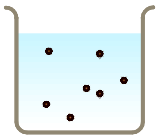
Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 30 minutes.**

IMPORTANT! Perform a standard curve with each assay.



Add sample

Add 50 µL of standards or diluted samples (see page 2) to the appropriate wells.



Add chromogenic detection reagent

a. Add 100 µL Creatinine Reagent into each well.
b. Tap the side of the plate to mix.
c. Incubate for 30 minutes at room temperature.

Read the plate and generate the standard curve

1. Read the absorbance at 490 nm.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than that of the highest standard in distilled or deionized water and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

The following data were obtained for the various standards over the range of 0–20 mg/dL creatinine.

Standard Creatinine (mg/dL)	Optical Density (490 nm)
20	2.315
10	1.296
5	0.703
2.5	0.423
1.25	0.270
0.625	0.200
0.313	0.163
0	0.129

Intra-assay precision

Four human urine samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3	Sample 4
Mean (mg/dL)	8.92	4.08	1.94	1.11
%CV	2.8	1.3	2.5	3.0

CV = Coefficient of Variation

Inter-assay precision

Four human urine samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3	Sample 4
Mean (mg/dL)	9.04	4.18	2.03	1.18
%CV	2.3	2.7	3.9	3.7

CV = Coefficient of Variation

Performance characteristics, continued

Expected values

Random urine samples were evaluated for the presence of creatinine with this assay.

Sample	Range (mg/dL)	Average (mg/dL)
Human (n=47)	17.2–168.9	90.7
Beagle (n=1)	—	92.8
Rat (n=1)	—	25.2
Rhesus monkey (n=1)	—	2.65

Interferents

A diluted urine sample was spiked with 2,000 mg/dL of glucose (equivalent to 40,000 mg/dL undiluted) and tested with the kit. The unspiked diluted sample read at 8.44 mg/dL. No significant change to the measured creatinine level was seen at any glucose concentration.

Linearity of dilution

Linearity was determined by assaying high and low concentration human urine samples (high sample 9.33 mg/dL; low sample 0.38 mg/dL) mixed in the ratios shown in the following table.

High Sample %	Low Sample %	Expected Conc. (mU/mL)	Observed Conc. (mU/mL)	% Recovery
80	20	7.54	7.75	102.8
60	40	5.75	5.89	102.4
40	60	3.96	4.12	104.0
20	80	2.17	2.29	105.5
			Mean Recovery	**103.7%**

Sensitivity

The analytical sensitivity of the assay is 0.019 mg/dL creatinine. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Limited product warranty

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Catalog Number

Batch code


Temperature limitation


Use by


Manufacturer


Consult instructions for use


Caution, consult accompanying documents

Manufacturer's address: Life Technologies Corporation | 7335 Executive Way | Frederick, MD 21704 | USA

The information in this guide is subject to change without notice.

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