CREATIVE BIOMART®
Assay Kit

Urinary Creatinine Colorimetric Assay Kit

Product Information

Common Name

Urinary Creatinine

Cat.No. Kit-2531

Product Overview

Urinary Creatinine Colorimetric Assay Kit can be used to measure creatinine levels in urine. The assay relies on the Jaffe' reaction, wherein a yellow/orange color forms when the metabolite is treated with alkaline picrate. The color derived from creatinine is then destroyed at acidic pH. The difference in color intensity measured at 500 nm before and after acidification is proportional to the creatinine concentration. The sample creatinine concentration is determined using a creatinine standard curve.

Description

Creatine synthesized in kidney, liver, and pancreas is transported in blood to muscle and brain where it is phosphorylated to phosphocreatine. Some free creatine in muscle is converted to creatinine. The amount of creatinine produced is proportional to the individuals muscle mass. In the absence of renal disease, the excretion rate of creatinine in an individual is relatively constant. Thus, urinary creatinine levels may be used as an index of standardization for other tests. Measurement of creatinine clearance is also useful in detecting renal disease and estimating the extent of impairment of renal function.

Storage

Store the Creatinine Standard at 4°C and the rest of the kit at room temperature (18-26°C).

Kit Components

Creatinine Standard, 1 vial/3 ml
Creatinine Color Reagent, 1 vial/12 ml
Creatinine Sodium Hydroxide, 1 vial/5 ml
Creatinine Acid Solution, 1 vial/1 ml

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Creatinine Sodium Borate, 1 vial/2.5 ml
Creatinine Surfactant, 1 vial/7.5 ml
96-Well Solid Plate(Colorimetric Assay), 1 plate
96-Well Cover Sheet, 1 cover

Materials Required but Not Supplied

1. A plate reader capable of measuring absorbance between 490-500 nm
2. Adjustable pipettes and a repeating pipettor
3. A source of pure water; glass distilled water or HPLC-grade water is acceptable

Technical Notes

General Information

- The final volume of the assay is 170 µl in all wells.
- All reagents except samples must be equilibrated to room temperature before beginning the assay.
- It is not necessary to use all the wells on the plate at one time.
- If the concentration of creatinine in the sample is not known or if it is expected to be beyond the range of the standard curve, it is prudent to assay the sample at several dilutions.
- It is recommended that the standards and samples be assayed at least in duplicate (triplicate is recommended).
- Monitor the absorbance at 490-500 nm using a plate reader.

Detection method Colorimetric

Preparation

Reagent Preparation

1. Creatinine Standard

The Creatinine Standard contains 20 mg/dl of creatinine in water. It is ready to use to prepare the standard curve. Sufficient Creatinine Standard is provided to prepare two standard curves using the 3 ml size or ten standard curves using the 15 ml size.

2. Creatinine Color Reagent

The color reagent contains 1.2% picric acid. The picric acid may contain crystals. This is normal and will disappear upon making the Alkaline Picrate Solution.

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3. Creatinine Sodium Hydroxide
The vial contains 1 M sodium hydroxide (NaOH). It is ready to use as supplied.
4. Creatinine Acid Solution
The acid solution contains a mixture of sulfuric and acetic acid. It is ready to use as supplied.
5. Creatinine Sodium Borate
The vial contains a solution of sodium borate. It is ready to use as supplied.
6. Creatinine Surfactant
The vial contains a solution of surfactant. It is ready to used as supplied.
7. Alkaline Picrate Solution
The volume of Alkaline Picrate Solution needed is dependent on the number of wells being assayed. Calculate 150 µl for each well (i.e., To prepare sufficient reagent for one 96-well plate, mix together 2 ml of Creatinine Sodium Borate, 6 ml of Creatinine Surfactant, 10 ml of Creatinine Color Reagent, and 3.6 ml of Creatinine NaOH). The Alkaline Picrate Solution is stable for at least one week stored in the dark at room temperature.

Sample Preparation
Urine
Typically, human urine has creatinine levels in the range of 25-400 mg/dl (one time collection) or 500-2,000 mg/24 hours.
1. Collect urine in a clean container and store on ice. If not assaying on the same day, freeze the sample at -80°C. The sample will be stable for at least one month.
2. If a 24 hour urine sample is desired, collect the total volume of urine over a 24 hour period. Store the pooled urine at 4°C until all the collections are taken. If not assaying after all the collections are taken, freeze 5 ml of the pooled 24 hour collection at -80°C. The sample will be stable for at least one month.
3. Urine should be diluted 1:10 or 1:20 with HPLC-grade water before assaying.
NOTE: The Creatinine (urinary) Colorimetric Assay is not recommended for plasma or serum samples. Precipitation may occur in the wells upon the addition of the acid solution.

Standard Preparation

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For the determination of creatinine in urine, prepare the Creatinine Standards according to Table 1, below. Take eight clean glass test tubes and label them A-H. Add the amount of Creatinine Standard and HPLC-grade water to each tube as described in Table 1, below.

Tube Creatinine Standard (µl) HPLC-grade water (µl) Final concentration (mg/dl creatinine)
A 0 500 0
B 50 450 2
C 100 400 4
D 150 350 6
E 200 300 8
F 250 250 10
G 300 200 12
H 375 125 15

Table 1. Concentration of Standards

Assay Protocol

1. Creatinine Standard Wells - Add 15 µl of Creatinine Standard (tubes A-H) per well in the designed wells on the plate.
2. Sample Wells - Add 15 µl of sample to two wells. To obtain reproducible results, creatinine levels from each sample should fall within the absorbance values of the standard curve. When necessary, samples can be diluted with HPLC-grade water to bring the creatinine concentration to this level.
3. Initiate the reactions by adding 150 µl of Alkaline Picrate Solution to all the wells being used.
4. Cover the plate with the plate cover and incubate on a shaker for 10 minutes at room temperature.
5. Remove the plate cover and read the absorbance at 490-500 nm using a plate reader. This absorbance is the Initial absorbance reading (Iabs).
6. Add 5 µl of acid solution to all of the wells being used.
7. Cover the plate with the plate cover and incubate on a shaker for 20 minutes at room temperature.
8. Remove the cover and read the absorbance at 490-500 nm using a plate reader. This absorbance is the Final absorbance reading (Fabs).

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Analysis

1. Calculate the average Initial absorbance (Iabs) of each standard and sample.
2. Calculate the average Final absorbance (Fabs) of each standard and sample.
3. Subtract the average Final absorbance from the average Initial absorbance. This is your Corrected absorbance.
4. Subtract the average Corrected absorbance of standard A from itself and all other standards and samples. This is the adjusted absorbance.
5. Plot the adjusted absorbance of the standards (from step 4 above) as a function of the final concentration of creatinine from Table 1.
6. Calculate the creatinine concentration of the samples using the equation obtained from the linear regression of the standard curve substituting adjusted absorbance values for each sample.

Creatinine (mg/dl) = [Sample absorbance - (y-intercept)] /Slope x Sample dilu□on

NOTE: To convert the results from mg/dl to µmol/l, multiply the creatinine concentration (mg/dl) by 88.4.

Sensitivity

Under the standardized conditions of the assay described in this booklet, the dynamic range of the kit is 0-15 mg/dl of creatinine.

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