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CREATININE

CMA KIT INSTRUCTIONS PRODUCT #133319
FORENSIC USE ONLY

INTENDED USE: For the determination of Creatinine levels in human urine.

DESCRIPTION

Neogen Corporation's Creatinine CMA (Colorimetric Microplate Assay) test kit is a quantitative one-step kit designed for use as a quantitative analysis of creatinine levels in urine. The kit was designed for screening purposes and is intended for forensic use only.

ASSAY PRINCIPLES

Urinary Creatinine metabolite reacts with picric acid under alkaline conditions to produce an orange color which can be quantified by absorption spectroscopy near 500 nm wavelength. The Jaffe reaction also occurs non-specifically with other components in biological fluids. The specific reaction color produced with Creatine is, however, known to degrade rapidly under acidic conditions (Slot *et al*.). Heinegard and Tiderstrom showed that the difference in color intensity determined before and after addition of acid is a direct estimate of creatinine concentration.

First, samples and standards are added to the optically clear microplate. Next, a one part to five parts mixture of alkaline solution to yellow picric acid is added and incubated at room temperature for 10 minutes. During this incubation, the creatinine metabolite is reacting with the picric acid/alkaline solution mixture to produce an orange color. After the completion of the incubation, the plate is read at 490 nm for the first time. Following the reading of the first plate, acetic acid is added to the plate and incubated at room temperature for five minutes. After incubation, the plate is read at 490 nm for the second time. After reading the second plate, the assay is completed. To determine the creatinine concentration of the sample, subtract the second plate read ODs from the first plate read ODs and compare the final ODs to those of the standards.

STORAGE AND STABILITY

This kit can be used until the expiration date on the label when stored properly. Standards (S1, S2, and S3) and EIA Buffer must be stored at 2-8°C whereas all other kit components must be stored at room temperature. The kit is stable until the expiration date listed on the kit label.

MATERIALS PROVIDED – SINGLE KIT (96 WELL)

1. **EIA Buffer:** 2 x 30 mL. Phosphate buffered saline solution with bovine serum and a preservative. Provided for dilution of samples. Ready to use.
2. **Creatinine Standards:** 110 µL of each standard. Standard 1 (S1) is a 10 mg/dL creatinine standard. Standard 2 (S2) is a 3 mg/dL creatinine standard. Standard 3 (S3) is a 1 mg/dL creatinine standard. Ready to use.
3. **R1: Yellow Reagent:** 20 mL. 0.6% Picric acid in sodium borate buffer. Must be combined with R2: Alkali solution in a five parts to one part ratio prior to use.
4. **R2: Alkali Solution:** 4 mL. 1N NaOH (Sodium Hydroxide). Must be combined with R1: Yellow Reagent in a one part to five parts ratio prior to use.
5. **R3: Acid Reagent:** 1.5 mL. Acetic acid mixture. Ready to use. Do not dilute.
6. **Microplate:** A 96 well Nunc microplate with 8 breakaway strips.

MATERIALS NEEDED BUT NOT PROVIDED

1. Deionized water for Standard 4 (S4) (0.0 mg/dL Creatinine standard).
2. Precision pipettes that range from 10 µL – 1000 µL and disposable tips.
   Note: If all or several strips are to be used at one time, it is suggested that a multichannel pipette be used.
3. Clean test tubes for dilution of samples.
4. Microplate reader with 490 nm filter.
5. Plate cover or plastic film to cover plate during incubation.
6. Microplate shaker.

PRECAUTIONS AND NOTES

1. **DO NOT** use kits or components beyond expiration date.
2. **DO NOT** mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
3. **DO NOT** pipette reagents by mouth.
4. All specimens should be considered potentially infectious. Exercise proper handling precautions.
5. Keep plate covered except when adding reagents or reading.
6. Standards should be refrigerated at all times when not in use.
7. Use aseptic technique when opening and removing reagents from vials and bottles.
8. **DO NOT** smoke, eat or drink in areas where specimens or reagents are being handled.
9. All kit components except standards should be stored at room temperature (20-26°C). Turbidity develops in picric acid at lower temperatures and this effect may be removed by warming. Reagent is still usable after being warmed.
10. Picric Acid (yellow color reagent) can be explosive when dry. Can irritate the eyes, skin, and the respiratory system. Wear suitable protective clothing, gloves, and eye shield when handling this reagent.

SAMPLE TREATMENT

Recommended minimum sample dilutions are listed below. These dilutions may change based on your laboratory's determination. All sample dilutions should be made in Neogen's EIA Buffer.

a. Urine: A 1:5 dilution is required for optimal assay performance.

TEST PROCEDURES

The following test procedures can be run manually or on an automated instrument. Please contact your Neogen representative for assistance with protocols for automated instruments.

1. Obtain approximately 100 µL of deionized water and place into a test tube for use as Standard 4 (S4) 0.0 mg/dL.
2. Add 25 µL of standards and diluted samples in duplicate to the appropriate wells of the microplate.
3. Add the contents of the R2 Alkali solution to the R1 Yellow Picric Acid solution. If the entire plate is not to be used at once, mix the reagents in a one part to five parts ratio.
4. Add 180 µL of the alkaline/picric acid solution to each well. (Use a multichannel pipette for rapid addition).
5. Mix contents by shaking plate gently (A microplate shaker may be used).
6. Cover plate with plastic film or plate cover and incubate at room temperature for 10 minutes.
   Note: Keep plate away from drafts and temperature fluctuations.
7. Perform the first plate reading at 490 nm.
8. Add 15 µL of R3: Acid reagent to each well. (A multichannel pipette may be used for rapid addition).
9. Mix contents thoroughly by placing plate on a microplate shaker for 60 seconds.
10. Cover plate with plastic film or plate cover and incubate at room temperature for 5 minutes.
11. Perform the second plate reading at 490 nm.

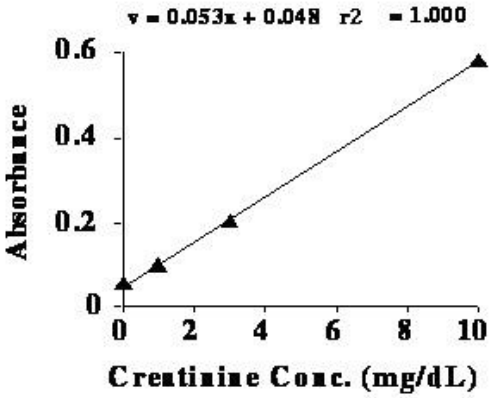
CREATININE CONCENTRATION CALCULATIONS

Calculations are required in order to determine the creatinine concentrations in the standards and samples. The following steps outline the calculation process. Please contact your Neogen representative for assistance with the creatinine concentration calculations.

1. Subtract the ODs of the second plate read from the ODs of the first plate read to determine 'Final ODs' for all standards and samples tested. The difference is absorbance between the second and first plate reads (Final ODs) is directly proportional to the creatinine concentration in the sample.
   Note: Some microplate readers can be programmed to perform these subtractions automatically when reading the plate. Consult the instrument manual for further details.
3. Construct an x,y axis linear graph where creatinine concentration (range of 0 mg/dL to 10 mg/dL) is on the x-axis and the difference in absorbance (range of 0 to 0.800) is on the y-axis.

4. Plot the Final OD readings of the four standards tested on the graph. Once the points are plotted, connect all points with a line (Linear trend line should NOT be used).
5. Plot the Final ODs of the samples on the standard line in the spot that corresponds with the Final OD reading.
6. The sample's creatinine concentration is wherever the point intersects with the x-axis.
7. Once this value is determined, multiply the value by the dilution factor of 5 to obtain the final creatinine concentration.

TYPICAL STANDARD CURVE



INTERFERING SUBSTANCES

Urine containing bilirubin will give elevated creatinine results. Additionally, this creatinine concentration measurement is not useful with urine samples that contain sulfonphthalein dyes such as phenosulfonphthalein as certain drugs are known to interfere with circulating creatinine levels, and hence will not provide consistent results (Young, 1990).

REFERENCES

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4. Young, DS, editor. Effects of drugs on clinical laboratory tests. AACC Press, Washington. 1990.
5. Wyss, M and Kaddurah-Daouk, R. Creatine and Creatinine Metabolism. Physiological Reviews. 2000, 80:1107.

TECHNICAL SUPPORT

For technical assistance, please contact our Technical Services Department at (859) 254-1221 or email at techservice-toxicology@neogen.com. Representatives are available Monday – Friday from 8:00 am – 6:00 pm EST.

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D13319-01/25/18