Colorimetric Microplate Assay for

CREATINE

Product No. 430210

For Research Use Only

INTRODUCTION

Background

Creatine (Cr) produced in the kidney, liver and pancreas is transported to the brain and muscle and phosphorylated. However, a small proportion of free Cr is converted irreversibly to creatinine (Crn) in the muscular tissue in proportion to the muscle mass. The amount of Crn excreted daily by an individual is relatively constant. Thus, urinary creatinine levels may be used as an index of standardization. Also, since the rate of nonenzymatic production of Crn from Cr is nearly constant and because >90% of the total bodily Cr is found in muscle tissue, 24-h urinary Crn excretion is used as an estimate of total muscle mass. Normal urinary creatinine values for men and women range from 9.7 – 24.7 and 7.9 – 14.2 mmol/24h respectively. Changes in excretion rate may be indicative of impaired renal metabolism.

Principles of the Assay

This is a colorimetric assay for the quantitative analysis of creatinine levels in urine. 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 however, occurs non-specifically with other components in biological fluids also. 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.

REAGENTS

MATERIALS PROVIDED:

1.	**S1: Standard 1**	(10mg/dl)	0.05 ml
2.	**S2: Standard 2**	(3mg/dl)	0.100 ml
3.	**R1: Yellow Reagent**	Picric acid, 0.6% in sodium borate buffer	20.0 ml
4.	**R2: Alkali solution**	1 N NaOH,	4.0 ml
5.	**R3: Acid Reagent**	Mixture of sulfuric and acetic acids.	1.0 ml
6.	**Microplate:**	A 96 well strip well microplate.	

MATERIALS NEEDED BUT NOT PROVIDED:

1. Deionized water for diluting urine samples and standards.
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 used to dilute the standards.
4. Microplate reader with 490/500 nm filter.
5. Plastic film or plate cover to cover plate during incubation.
6. Plate Shaker

WARNINGS AND PRECAUTIONS:

1. **DO NOT** use 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. Always pour substrate out of the bottle into a clean test tube - **DO NOT** pipette out of the bottle (if your tip is unclean you could contaminate your substrate).
5. All specimens should be considered potentially infectious. Exercise proper handling precautions.
6. **DO NOT** smoke, eat or drink in areas where specimens or reagents are being handled.
8. Keep plate covered except when adding reagents, washing or reading.
9. All kit components *except* standards should be stored at room temperature (20-26 °C). Turbidity developed in picrate reagent at lower temperatures may be removed by warming. Reagent is still usable.
10. Creatinine standard should be refrigerated (4 °C).
11. Picric Acid (yellow color reagent) can be explosive when dry. Can irritate the eyes, skin and the respiratory system. Wear suitable protective clothing, gloves, eye shield.

TEST PROCEDURE

1. Prepare standards as follows:

   S1 Standard solution 10 mg/dL (provided)

   S2 Standard solution 3 mg/dL (provided)

   S3 Add 50 µL of S2, to 100 µL of deionized water. Mix (1 mg/dL standard)

   S4 Distilled water = 0.0 ng/mL.

2. Add 0.018 mL/well of standards (S) or unknowns (U) (some samples may require diluting) to the appropriate wells in duplicate. See **Scheme I** for suggested template design.

Scheme I

	1	2	3	4	5	6	7	8	9	10	11	12
A	S4	S4	U5	U5	U13	U13	U21	U21	U29	U29	U37	U37
B	S3	S3	U6	U6	U14	U14	U22	U22	U30	U30	U38	U38
C	S2	S2	U7	U7	U15	U15	U23	U23	U31	U31	U39	U39
D	S1	S1	U8	U8	U16	U16	U24	U24	U32	U32	U40	U40
E	U1	U1	U9	U9	U17	U17	U25	U25	U33	U33	U41	U41
F	U2	U2	U10	U10	U18	U18	U26	U26	U34	U34	U42	U42
G	U3	U3	U11	U11	U19	U19	U27	U27	U35	U35	U43	U43
H	U4	U4	U12	U12	U20	U20	U28	U28	U36	U36	U44	U44

3. Add the contents of the Alkali solution R2 to the Yellow Picric Acid solution R1. If the entire plate is not to be used at once, mix the solutions in a one part to five parts ratio.
4. Add 0.18 mL/well of the alkaline picrate reagent mixture (Use 8-channel pipette for rapid addition.)
5. Mix 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. Read plate at 490 nm (First Reading)
8. Add 0.006 ml/well of acid reagent.
9. Mix thoroughly by triturating (A microplate shaker may be used.)
10. Allow to stand at room temperature for 5 minutes.
11. Read plate again at 490 nm (Second reading)
12. Subtract the second set of absorbance readings from the first set.

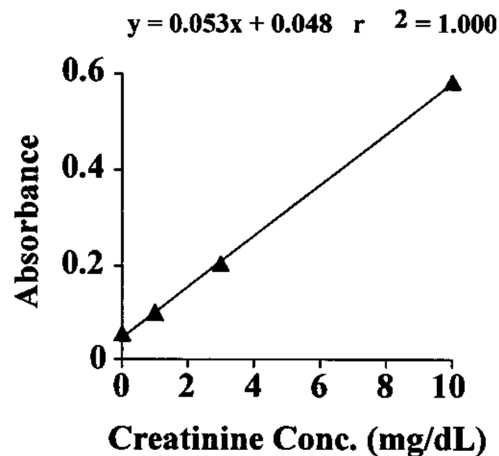
    NOTE: Some microplate readers can be programmed to do these subtractions automatically when reading the plate. Consult your instrument manual.

13. The difference in absorbance [Δ A] is directly proportional to Creatinine concentration.

14. Construct Standard Curve [Δ A on y axis vs conc (mg/dL) on x axis]. Determine creatinine concentration (mg/dL) in urine samples. Multiply the creatinine concentration in mg/dL by 88.4 to convert into µmol/L (SI unit).

    NOTE: Normally a 5-10 fold urine dilution yields results in the linear range of the standard curve. If the samples are diluted, the concentration determined from the standard curve must be multiplied by the dilution factor.

TYPICAL STANDARD CURVE:



Interfering Substances:

Samples containing Bilirubin will give elevated results. The measurement is not useful in samples containing sulfonphthalein dyes such as phenolsulfonphthalein. Certain drugs are known to interfere with circulating creatinine levels and hence will not provide consistent results. (Young, 1990).

REFERENCES

1. Slot C. Plasma creatinine determination. A new and specific Jaffe reaction method. *Scand J. Clin. Lab. Invest.* 1965, **17**: 381
2. Heinegard, D. and Tiderstrom, G. Determination of serum creatinine by a direct colorimetric method. *Clin. Chim. Acta* 1973, **43**: 305.
3. Cook JGH. Factors influencing the assay of creatinine. *Ann Clin. Biochem.* 1975, **43**: 305.
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.

MATERIAL SAFETY DATA SHEET

PHYSICAL AND CHEMICAL DATA: Components are stable in closed containers under normal temperatures and pressures.

PRECAUTIONS: Gloves and lab coat should be worn at all times while performing this assay. Contents may be harmful if swallowed, inhaled or absorbed through the skin.

Picric acid is **explosive** when dry and can form sensitive explosive metallic compounds. Incompatible with metals, strong oxidizing agents, strong bases, reducing agents, heavy metals, and ammonia.

HEALTH HAZARDS: Individual components may cause skin irritation or be harmful if swallowed. Avoid contact with skin and eyes.

FIRST AID: Call a physician. If swallowed, give water or milk to dilute and induce vomiting. In case of contact with eyes, flush with copious amounts of water for at least 15 minutes. Assure adequate flushing by separating eyelids with fingers. In case of skin contact, wash with soap or mild detergent and large amounts of water.

ORDERING INFORMATION

For additional kits or a complete catalog please contact us.

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