

Microplate Assay for Creatinine

For Research Use Only

INTRODUCTION

Creatine (Cr) is produced in the kidney, liver and pancreas, phosphorylated, and transported to the brain and muscle tissue. 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. 24-h urinary Crn excretion is used to estimate total muscle mass because the rate of non-enzymatic production of Crn from Cr is nearly constant and >90% of the total body Cr is found in muscle tissue. 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 PROCEDURE

This is a colorimetric assay for the quantitative analysis of creatinine levels in urine. Urinary creatinine reacts with picric acid under alkaline conditions to produce an orange color, which can be quantified by absorption spectroscopy near the 500 nm wavelength. This reaction, known as the Jaffe reaction, also occurs non-specifically with other components in biological fluids. However, the specific color produced with creatine in this reaction is known to degrade rapidly under acidic conditions (Slot et al.). Heinegard and Tiderstrom showed that the difference in color intensity determined before and after the addition of acid is a direct estimate of creatinine concentration in the sample.

MATERIALS PROVIDED

Component	Description	Volume	Storage	Cat no.
S1: Creatinine Standard 1	Creatinine Standard Solution (10 mg/dL)	110 μ L	4°C	CR01a
S2: Creatinine Standard 2	Creatinine Standard Solution (3 mg/dL)	110 μ L	4°C	CR01b
S3: Creatinine Standard 3	Creatinine Standard Solution (1 mg/dL)	110 μ L	4°C	CR01c
R1: Picrate Reagent	Picric Acid Solution	20 mL	25°C	CR01d
R2: Alkali Solution	NaOH and Sodium Borate Solution	4 mL	25°C	CR01e
R3: Acid Reagent	Acetic Acid Solution	2 mL	25°C	CR01f
Microplate	96-well Microplate	1 plate	25°C	CR01g

MATERIALS NEEDED BUT NOT PROVIDED

1. Microplate reader with 490 or 500 nm filter
2. Adjustable micropipettes (10 – 1000 μ L) and tips
3. Deionized water
4. Plate shaker
5. Plate cover or plastic film

STORAGE

1. Store the components of this kit at the temperatures specified on the labels.
 2. Unopened reagents are stable until the indicated kit expiration date.
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WARNINGS AND PRECAUTIONS

1. Use aseptic technique when opening and dispensing reagents.
 2. This kit is designed to work properly as provided and instructed. Additions, deletions or substitutions to the procedure or reagents are not recommended, as they may be detrimental to the assay.
 3. Picric acid can be explosive when dry, and can irritate the eyes, skin and respiratory system. Wear suitable protective clothing, gloves, and eye protection.
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PROCEDURAL NOTES

1. Turbidity may develop in the Picrate Reagent at lower temperatures and may be removed by warming. The reagent is still usable.
 2. To minimize errors in absorbance measurements due to handling, wipe the exterior bottom of the microplate wells with a lint-free paper towel prior to inserting into the plate reader.
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REAGENT PREPARATION

1. **Alkaline Picrate Reagent:** Add R2: Alkali Solution to R1: Picrate Reagent in a one part to five parts ratio. If the entire plate is being used, add the entire contents of R2 to R1.
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STANDARD CURVE PREPARATION

The Creatinine Standards are provided ready to use. A null Standard (0 mg/dL) is made with deionized water.

Table 1: Standard Curve Preparation

Standard	Creatinine Concentration (mg/dL)	Volume of Water (µL)	Volume of Creatinine Standard (µL)
S ₁	10.0	-	110
S ₂	3.0	-	110
S ₃	1.0	-	110
S ₄	0	110	-

ASSAY PROCEDURE

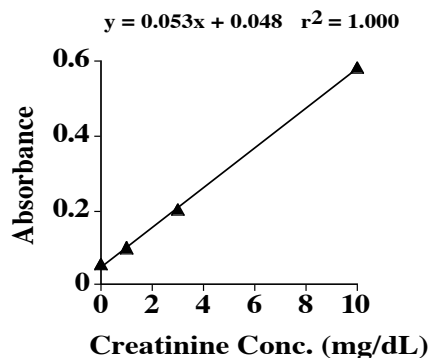
1. Add 25 µL of Standards or Samples (may require diluting 1:4 or 1:8) to the corresponding wells on the microplate in duplicate. See **Scheme I** for a sample plate layout.
2. Add 180 µL of the Alkaline Picrate Reagent to each well.
3. Mix by shaking or placing the plate on a shaker and incubate at room temperature for 10 minutes.
4. Read the plate at 490 nm. (First Reading)
5. Add 15 µL of R3: Acid Reagent to each well.
6. Mix thoroughly by tapping or shaking and allow to stand at room temperature for 5 minutes.
7. Read the plate again at 490 nm. (Second Reading)

Scheme I: Sample Plate Layout

□	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

CALCULATIONS

- Subtract the values of the Second Reading from those of the First Reading.
NOTE: Some microplate readers can be programmed to do these subtractions automatically when reading the plate. Consult your instrument manual.
- The difference in absorbance [ΔA] is directly proportional to Creatinine concentration.
- Construct a Standard Curve with ΔA on the y-axis versus Creatinine Conc. (mg/dL) on the x-axis.
- Determine the creatinine concentration (mg/dL) in the samples.
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.
- Multiply the creatinine concentration in mg/dL by 88.4 to convert into $\mu\text{mol/L}$ (SI unit).

Figure 1: 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

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- Cook, J.G.H.; (1975) *Ann Clin. Biochem.* **43**:305
- Young, D.S., Editor; (1990) Effects of drugs on clinical laboratory tests. AACC Press, Washington
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