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 μL	4°C	CR01a
S2: Creatinine Standard 2	Creatinine Standard Solution (3 mg/dL)	110 μL	4°C	CR01b
S3: Creatinine Standard 3	Creatinine Standard Solution (1 mg/dL)	110 μL	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 μL) 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.

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.

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.

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.

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)

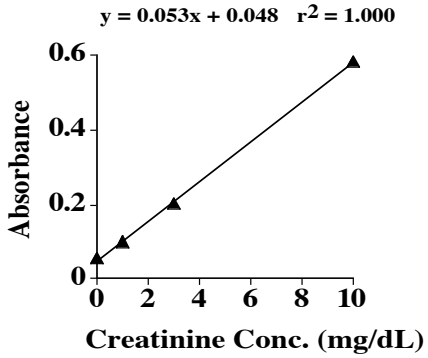
Scheme I: Sample Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S_4	S_4	U_5	U_5	U_{13}	U_{13}	U_{21}	U_{21}	U_{29}	U_{29}	U_{37}	U_{37}
B	S_3	S_3	U_6	U_6	U_{14}	U_{14}	U_{22}	U_{22}	U_{30}	U_{30}	U_{38}	U_{38}
C	S_2	S_2	U_7	U_7	U_{15}	U_{15}	U_{23}	U_{23}	U_{31}	U_{31}	U_{39}	U_{39}
D	S_1	S_1	U_8	U_8	U_{16}	U_{16}	U_{24}	U_{24}	U_{32}	U_{32}	U_{40}	U_{40}
E	U_1	U_1	U_9	U_9	U_{17}	U_{17}	U_{25}	U_{25}	U_{33}	U_{33}	U_{41}	U_{41}
F	U_2	U_2	U_{10}	U_{10}	U_{18}	U_{18}	U_{26}	U_{26}	U_{34}	U_{34}	U_{42}	U_{42}
G	U_3	U_3	U_{11}	U_{11}	U_{19}	U_{19}	U_{27}	U_{27}	U_{35}	U_{35}	U_{43}	U_{43}
H	U_4	U_4	U_{12}	U_{12}	U_{20}	U_{20}	U_{28}	U_{28}	U_{36}	U_{36}	U_{44}	U_{44}

CALCULATIONS

1. 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.
2. The difference in absorbance [Δ A] is directly proportional to Creatinine concentration.
3. Construct a Standard Curve with Δ A on the y-axis versus Creatinine Conc. (mg/dL) on the x-axis.
4. 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.
5. Multiply the creatinine concentration in mg/dL by 88.4 to convert into μmol/L (SI unit).

Figure 1: Typical Standard Curve



INTERFERING SUBSTANCES

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

REFERENCES

1. Slot, C.; (1965) *Scand J. Clin. Lab. Invest.* **17**:381
2. Heinegard, D. and Tiderstrom, G.; (1973) *Clin. Chim. Acta* **43**:305
3. Cook, J.G.H.; (1975) *Ann Clin. Biochem.* **43**:305
4. Young, D.S., Editor; (1990) Effects of drugs on clinical laboratory tests. AACC Press, Washington
5. Wyss, M. and Kaddurah-Daouk, R.; (2000) *Phys. Rev.* **80**:1107

DISCLAIMER

This information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. Oxford Biomedical Research, Inc. shall not be held liable for any damage resulting from handling or from contact with the above product. See catalog for additional terms and conditions of sale.

ORDERING INFORMATION

For additional kits or a complete catalog please call 800-692-4633.

TECHNICAL SUPPORT

If you need technical information or assistance with assay procedures, call our Technical Support Department at 800-692-4633 or 248-852-8815. Our staff will be happy to answer your questions about this or any other product in the Oxford Biomedical line.

GUARANTEE AND LIMITATION OF REMEDY

Oxford Biomedical Research, Inc. makes no guarantee of any kind, expressed or implied, which extends beyond the description of the materials in this kit, except that these materials and this kit will meet our specifications at the time of delivery. Buyer's remedy and Oxford Biomedical Research, Inc.'s sole liability hereunder is limited to, at Oxford Biomedical Research, Inc.'s option, refund of the purchase price of, or the replacement of, material that does not meet our specification. By acceptance of our products, Buyer indemnifies and holds Oxford Biomedical Research, Inc. harmless against, assumes all liability for the consequence of its use or misuse by the Buyer, its employees, or others. Said refund or replacement is conditioned on Buyer notifying Oxford Biomedical Research, Inc. within thirty (30) days of the receipt of product. Failure of Buyer to give said notice within thirty (30) days of receipt of product shall constitute a waiver by the Buyer of all claims hereunder with respect to said material(s).

Oxford Biomedical Research, Inc.
P.O. Box 522
Oxford, MI 48371 U.S.A.

Orders: 800-692-4633
Technical Service: 248-852-8815
Fax: 248-852-4466
E-mail: info@oxfordbiomed.com

Made in the U.S.A.