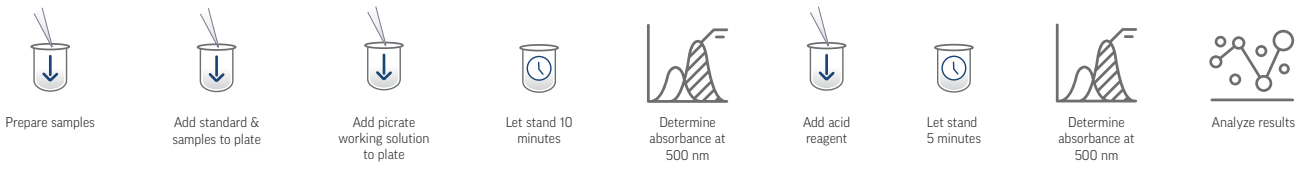
THE CREATININE COMPANION



DESCRIPTION

Creatinine, a waste product of creatine metabolism, is normally filtered from the bloodstream via the kidneys and excreted in urine. The Exocell Creatinine Companion is designed to ascertain the relative amount of creatinine in a sample in comparison to known concentrations of creatinine. This kit is meant as a companion to Exocell ELISA Kits for albumin (Albuwell M, Albuwell Hu, Nephrat II). By using this microwell plate format for determining the concentration of creatinine and albumin, a value for the albumin to creatinine ratio (ACR) can be determined simultaneously from the sample.

The procedure is an adaptation of the alkaline picrate method (1) and entails determination of the differential absorbance in a sample before and after the addition of acid to correct for color generation due to interfering substances (2).

KIT CONTENTS

1. 96-well microtiter plates (2)
2. Picrate Reagent (2 x 10 mL)
3. 0.5 M NaOH
4. Acid Reagent (2 x 12 mL)
5. Standards (1, 3, and 10 mg/dL)

ASSAY PROCEDURE

1. **Reagent Preparation:** Add 2.0 mL of 0.5 M NaOH to a bottle of 10 mL Picrate Reagent. This is a working solution of alkaline picrate (picrate working solution) and must be used immediately after preparation.
2. **Sample Dilution:** The appropriate dilution will depend on sample origin and the method of collection. We suggest starting with 1:20 dilution in distilled water using disposable microfuge tubes, but further optimization may be required to ensure experimental samples are within the linear range of this kit. For dilutions and a plate map, please see Appendix.
3. Add 20 µL of water to wells A1 and A2. These are control blank wells.
4. Add 20 µL of Creatinine Standard, 10 mg/dL to wells A3 and A4.
5. Add 20 µL of Creatinine Standard, 3 mg/dL to wells A5 and A6.
6. Add 20 µL of Creatinine Standard, 1 mg/dL to wells A7 and A8.
7. Add a 20 µL aliquot of diluted sample to wells A9 and A10.
8. Continue the addition of sample aliquots to the rest of the plate.
9. Add 100 µL picrate working solution to each of the wells.
10. Incubate for 10 minutes on the bench top.
11. Determine the absorbance of the wells on a plate reader set at 500 nm. Well A1 serves as “Blank”.
12. Add 100 µL of Acid Reagent to each of the wells.
13. Incubate for 5 minutes.
14. Measure absorbance as described in Step 11.

DATA ANALYSIS

1. For the creatinine standard dilutions and the experimental samples, calculate the change in absorbance with this formula: ΔAbs = Abs(alkaline picrate) – Abs(alkaline picrate with acid)
2. Plot the concentration for Creatinine Standard on the x-axis and ΔAbs on the y-axis. Determine the least squares regression line. Do not include the blank.
3. Using the least squares regression line, determine the concentration of diluted samples based on the ΔAbs value.
4. Multiply these values by the reciprocal of the dilution factor to obtain concentration (mg/dL) in undiluted samples.

REFERENCES

1. Murray, RL: in Methods in Clinical Chemistry, AJ Pesce and LA Kaplan, ed, CV Mosby Co, St. Louis, pp 10-17, 1987.
2. Heinegard,D and Tiderstrom, G. Determination of serum creatinine by a direct colorimetric method. *Clin Chim Acta* 43:305. 1973 Rev. 3.2 09 January 2007

PRODUCT INFORMATION

CAT. #	DESCRIPTION
1012	The Creatinine Companion
1011	Albuwell M (mouse albumin)
1004	Albuwell Hu (human albumin)
NR002	Nephrat™ II (rat albumin)

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APPENDIX

SAMPLE PREP

For a 1:20 dilution

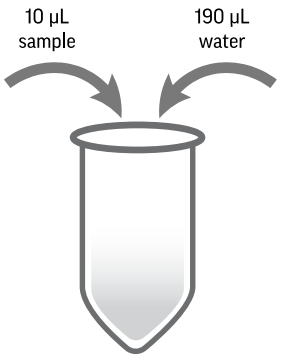

PLATE MAP

Add 20 µL of each of the following to the appropriate wells:

Key
W = water
C = Creatinine Standard
S# = Test samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	W	W	10 mg/dL C	10 mg/dL C	3 mg/dL C	3 mg/dL C	1 mg/dL C	1 mg/dL C	S1	S1	S2	S2
B	S3	S3	S4	S4	(etc...)							
C												
D												
E												
F												
G												
H												