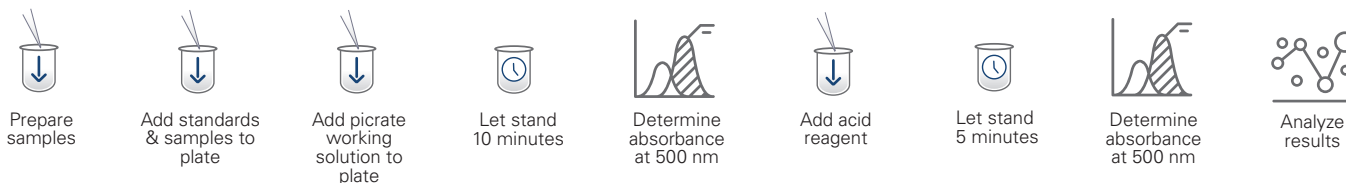


## THE CREATININE COMPANION



### DESCRIPTION

This assay is designed for companion use with Exocell's immunospecific ELISAs for urine albumin excretion\*, allowing expression of results as ug albumin per mg creatinine in the urine. Adaptation of creatinine measurement to microtiter wells enables convenient, simultaneous determination of albumin and creatinine concentrations in the same specimen, using analogous microtiter plate formats. Normalization of albumin excretion in relation to creatinine facilitates classification according to defined ranges of microalbuminuria, the established marker of early diabetic renal dysfunction (1-3). The urine albumin to creatinine ratio in a random urine specimen is an accepted alternative to cumbersome 24-hour urine collections in the detection and monitoring of microalbuminuria (4). The procedure is an adaptation of the alkaline picrate method (5), 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 (6).

### KIT CONTENTS

- 96 well microtiter plates (2)
  - Picrate Reagent (2x10ml)
  - 1 N NaOH
  - Acid Reagent (2x12 ml)
  - Standards (1, 3 and 10 mg/dL)
- \*Albuwell® for human albumin; Nephrat™ for rat albumin; AlbuwellIM™ for mouse albumin

### ASSAY PROCEDURE

1. **Reagent Preparation:** Add 2.0 mL of 1 N 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:** For human samples, prepare 1:20 dilution in distilled water in disposable microfuge tubes. For animal samples, appropriate dilution will depend on method of collection. If washing of collection apparatus is employed, further dilution may not be required. Otherwise, a 1:20 dilution is acceptable.
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. Let stand for 10 minutes on the bench top.
11. Determine the absorbance of the wells on a plate reader set at approximately 500 nm. Well A1 serves as "Blank".
12. Add 100 µL of Acid Reagent to each of the wells.
13. Let stand for 5 minutes.
14. Measure absorbance as described above.

## DATA ANALYSIS

1. Calculate the data absorbance:  $\Delta_{\text{alkaline picrate}} = \text{Absorbance alkaline picrate} - \text{Absorbance alkaline picrate} + \text{acid}$
2. Determine the least squares regression line using delta absorbance versus creatinine concentration for each standard. Do not include blank.
3. Determine the concentration of diluted samples by substituting the respective delta absorbances appropriately.
4. Multiply these values by the reciprocal of the dilution factor to obtain concentration (mg/dL) in undiluted samples.

## EXPECTED VALUES

The following albumin/creatinine ratios, taken from Warram et al (4), are provided as a guide for classification of microalbuminuria in human subjects.

### Normal: ug Albumin/mg creatinine

Men <17

Women <25

### Low Microalbuminuria:

Men 17-58

Women 25-63

### High Microalbuminuria:

Men 58-250

Women 63-355

### Clinical Proteinuria:

Men >250

Women >355

## REFERENCES

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Revision 4..5 June 2022

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## PRODUCT INFORMATION

Cat. #	Description
1012	The Creatinine Companion

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