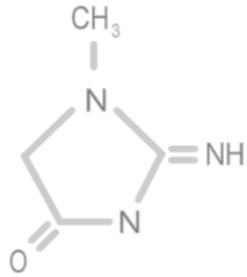
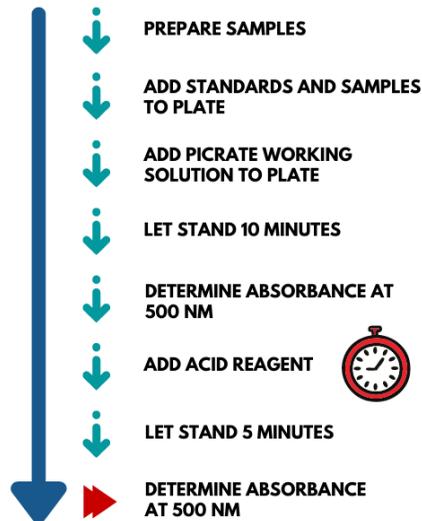


The Creatinine Companion by Exocell



CREATININE COMPANION WORKFLOW



RESULTS



2070 Center Square Road, Logan Township, NJ 08085

phone: (856) 224-0900

Email: service@ethosbiosciences.com

This assay is designed for companion use with Exocell's immunospecific ELISAs for urine albumin excretion *, allowing expression of results as μg 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; Nephtrat™ for rat albumin; AlbuwellIM™ for mouse albumin

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:
 $\Delta = \text{Alkaline picrate} - \text{Alkaline picrate} + \text{acid}$
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: μg 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

1. Mogensen, CE: Microalbuminuria is a predictor of clinical diabetic nephropathy. *Kidney Int* 31:673-689, 1987.
2. Mogensen, CE: Microalbuminuria predicts clinical proteinuria and early mortality in maturity-onset diabetes. *N Engl J Med* 310:356-369, 1984.
3. Mogensen, CE: Prediction of clinical diabetic nephropathy in IDDM patients: alternatives to microalbuminuria. *Diabetes* 39:761-767, 1990.
4. Warram, JH, Gearin, G, Laffel, L, Krolewski, AS: Effect of duration of Type I diabetes on the prevalence of stages of diabetic nephropathy defined by urinary albumin/creatinine ratio. *J Am Soc Nephrol* 7:930-937, 1996.
5. Murray, RL: in *Methods in Clinical Chemistry*, AJ Pesce and LA Kaplan, ed, CV Mosby Co, St. Louis, pp 10-17, 1987.
6. 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