Technical Manual

ChromaDazzle Protein Creatinine Ratio Assay Kit

Catalogue Code: BA0055

Pack Size: 100 assays

Research Use Only

DESCRIPTION

PROTEIN is filtered out of urine by the glomeruli of the kidneys. Albumin is the most common serum protein, thus the majority of the protein in urine is albumin. A damaged kidney will allow some protein through into the urine, the less protein in urine the better. Elevated protein levels in urine is called microalbuminuria or proteinuria, which typically arises due to type 1 diabetes, type 2 diabetes, or high blood pressure.

CREATININE is synthesized in the body at a fairly constant rate from creatine. In healthy individuals, creatinine secretion is independent of diet and is fairly constant. The creatinine clearance test has become one of the most sensitive tests for measuring glomerular filtration rate.

PROTEIN/CREATININE RATIO (PCR) remains the simplest and most convenient test for proteinuria. Other methods such as 24 hour urine test or timed urine test require strict adherence to sample collection protocol. Since the protein concentration is normalized to creatinine secretion, the urine sample can be taken at anytime and no diet or liquid restrictions are necessary for sample collection.

KEY FEATURES

Sensitive and accurate. Use 20 µL samples. Linear detection range in 96-well plate: 1 - 20 mg/dL Protein and 1 – 150 mg/dL Creatinine.

Fast and convenient. No sample pre-treatment is needed. Simple 10- minute "add-incubate-read" procedure.

High-throughput adaptable. The procedure can be readily automated for processing thousands of samples per day.

APPLICATIONS

Direct Assays: Protein creatinine ratio determination in urine samples (rat, mouse, human, not species specific).

Drug Discovery/Pharmacology: effects of drugs on protein and creatinine concentration, metabolism, and excretion.

KIT CONTENTS (100 tests in 96-well plates)

PR Reagent:	24 mL	**CR Reagent A**	6 mL
CR Reagent B:	6 mL	**Standard:**	1 mL

Storage conditions. The kit is shipped at room temperature. Store all reagents at 2-8°C. Shelf life: 12 months after receipt.

Precautions: Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURE

Samples can be analyzed immediately after collection, or stored in aliquots at 4°C or –20°C for 7 days. Avoid repeated freeze-thaw cycles. If particulates are present, centrifuge sample and use the clear supernatant for the assay. Equilibrate all components to room temperature.

Protein Determination

1. Samples are run in duplicate. Transfer 20 µL of each sample into four separate wells: two Sample wells and two Internal Standard wells.

   Add 5 µL dH_2O to Sample wells, and 5 µL of Standard to the Internal Standard wells.

   Transfer 25 µL of dH_2O into two wells. This will be the Blank in duplicate. *Note: Each sample does not require a separate Blank: the same Blank value can be used for all samples on a particular plate.*
2. Add 200 µL of PR Reagent to each protein determination wells.
3. Incubate 10 min at room temperature, and then read the optical density at 600 nm for Protein.

Note: if the $OD_{STANDARD}$ - OD_{SAMPLE} for a particular sample is lower than 0.05, dilute sample with an equal volume of water and repeat the assay. Multiply result by the dilution factor (2). A low internal standard signal is due to interference with other molecules in urine, dilution will decrease the interference allowing for proper measurement of protein level.

Creatinine Determination

1. Samples are run in duplicate. Transfer 20 µL of each sample into four separate wells: two Sample wells and two Internal Standard wells.

   Add 5 µL dH_2O to Sample wells, and 5 µL of Standard to the Internal Standard wells.

   Transfer 25 µL of dH_2O into two wells. This will be the Blank in duplicate. *Note: Each sample does not require a separate Blank: the same Blank value can be used for all samples on a particular plate.*

2. Prepare sufficient Working Reagent (WR) for all wells by mixing, for each creatinine determination well, 50 µL CR Reagent A, 50 µL CR Reagent B, and 150 µL dH_2O. Transfer 200 µL of WR into each creatinine determination well. *Note: Working Reagent is stable for 2 hours, we recommend making fresh reagents for each assay run.*

3. Incubate 10 min at room temperature, and then read the optical density at 530 nm for Creatinine determination.

Note: if the $OD_{STANDARD} - OD_{SAMPLE}$ for a particular sample is lower than 0.1, dilute sample with an equal volume of water and repeat the assay. Multiply result by the dilution factor (2). A low internal standard signal is due to interference with other molecules in urine, dilution will decrease the interference allowing for proper measurement of creatinine.

CALCULATION

Protein concentration of a Sample is calculated as

$$\textbf{[Protein]} = \frac{OD_{SAMPLE} - OD_{BLANK}}{OD_{STANDARD} - OD_{SAMPLE}} \times 10000 \times n \quad \textbf{(µg/dL)}$$

Creatinine concentration of a Sample is calculated as

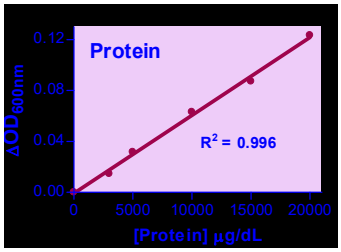
$$\textbf{[Creatinine]} = \frac{OD_{SAMPLE} - OD_{BLANK}}{OD_{STANDARD} - OD_{SAMPLE}} \times 25 \times n \quad \textbf{(mg/dL)}$$

Protein Creatinine Ratio of a Sample is calculated as

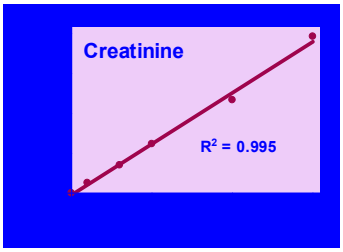
$$\textbf{Protein Creatinine Ratio} = \frac{\text{[Protein]}}{\text{[Creatinine]}} \quad \textbf{(µg/mg)}$$

where OD_{SAMPLE}, $OD_{STANDARD}$, and OD_{BLANK} are the optical density values of the Sample, Internal Standard, and Blank wells, respectively. 10,000 µg/dL and 25 mg/dL are the effective concentrations of the protein and creatinine Internal Standards respectively, and *n* is the dilution factor.

A Protein Creatinine Ratio of less than 30 is considered normal, from 30 – 300 is considered mild proteinuria (early kidney disease), and more than 300 indicates severe proteinuria (advanced kidney disease).



Protein Determination



Creatinine Determination

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), and plate or cuvette reader.

LITERATURE

1. Levey AS et al (2015). Glomerular filtration rate and albuminuria for detection and staging of acute and chronic kidney disease in adults: a systematic review. JAMA. 313(8):837-46.
2. Joern WA, Schmoele L (1981). Urinary protein measurement by the Coomassie blue dye-binding method adapted to the ABA-l00 bichromatic analyzer. Clin Chem27:1305.

3. Toora BD et al. (2002). Measurement of Creatinine by Jaffe's Reaction. Indian J Exp Biol. 40(3):352-4.

Contact Details

Dublin, Ireland

Email: info@assaygenie.com

Web: www.assaygenie.com

Technical Support: Techsupport@assaygenie.com