Technical Bulletin

Protein Creatinine Ratio Assay Kit

Catalog Number MAK445

Product 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. Elevated protein level 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.

Determination of the Protein/Creatinine Ratio (PCR) remains the simplest and most convenient test for proteinuria. Other methods such as the 24-hour urine test or timed urine test require strict adherence to sample collection protocol. Using the Protein Creatinine Ratio Assay Kit, protein and creatinine concentrations are determined separately and the protein concentration is normalized to the creatinine secretion. This allows urine samples to be taken at any time, and diet or liquid restrictions are not necessary prior to sample collection. The detection method for the kit is spectrophotometric, with protein measured at 600 nm and creatinine measured at 530 nm. The linear detection range for the method is 1 to 20 mg/dL protein and 1 to 150 mg/dL creatinine when analyzing 20 µL samples.

The Protein Creatinine Ratio Assay kit is suitable for the quantitative determination of urinary protein and creatinine concentrations (protein/creatinine ratio, UPCR) in urine samples.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- PR Reagent 24 mL
  Catalog Number MAK445A
- CR Reagent A 6 mL
  Catalog Number MAK445B
- CR Reagent B 6 mL
  Catalog Number MAK445C
- Standard 1 mL
  Catalog Number MAK445D

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at 2-8 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate components to room temperature prior to use.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

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.

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.
2. Add 5 µL of purified water to the Sample wells.
3. Add 5 µL of Standard to the Internal Standard wells.
4. Prepare duplicate Blank wells by transferring 25 µL of purified water into two wells.

   Note: The same Blank wells can be used for all Samples on a particular plate.

5. Add 200 µL of PR Reagent to each Sample, Internal Standard, and Blank Protein Determination well.
6. Incubate for 10 minutes at room temperature, then read the optical density (OD) at 600 nm for Protein Determination.
7. If the $OD_{STANDARD}$ - OD_{SAMPLE} for a particular sample is lower than 0.05, dilute the Sample with an equal volume of purified 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.
2. Add 5 µL of purified water to the Sample wells.
3. Add 5 µL of Standard to the Internal Standard wells.
4. Prepare duplicate Blank wells by transferring 25 µL of purified water into two wells.

   Note: The same Blank wells can be used for all Samples on a particular plate.

5. Mix enough reagents for the number of assays to be performed. For each Standard, Sample, Internal Standard and Water Blank well, prepare 250 µL of Working Reagent according to Table 1. Note: Working Reagent is stable for 2 hours. Prepare fresh reagent for each assay run.

Table 1.
Preparation of Working Reagent

Reagent	Working Reagent
CR Reagent A	50 µL
CR Reagent B	50 µL
Water	150 µL

6. Transfer 200 µL of Working Reagent into each Creatinine Determination well.
7. Incubate for 10 minutes at room temperature, and then read the optical density (OD) at 530 nm for Creatinine Determination.

8. If the $OD_{STANDARD}$ – OD_{SAMPLE} for a particular sample is lower than 0.1, dilute sample with an equal volume of purified 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.

Results

Note: The Optical Density readings used in the calculations are from the corresponding Procedure (Protein Determination at 600 nm and Creatinine Determination at 530 nm).

Protein (µg/dL) =

$$\frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times 10000 \times DF$$

Creatinine (mg/dL) =

$$\frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times 25 \times DF$$

Protein Creatinine Ratio (µg/mg) =

$$\frac{Protein\ (\mu g/dL)}{Creatinine\ (\frac{mg}{dL})}$$

where

OD_{Sample} = OD value of Sample

OD_{Blank} = OD value of Blank

$OD_{Standard}$ = OD value of Internal Standard

10000 = Protein concentration (µg/dL) of Internal Standard

25 = Creatinine concentration (mg/dL) of Internal Standard

DF = Sample Dilution Factor (DF = 1 for undiluted samples)

Figure 1.
Typical Protein Standard Curve

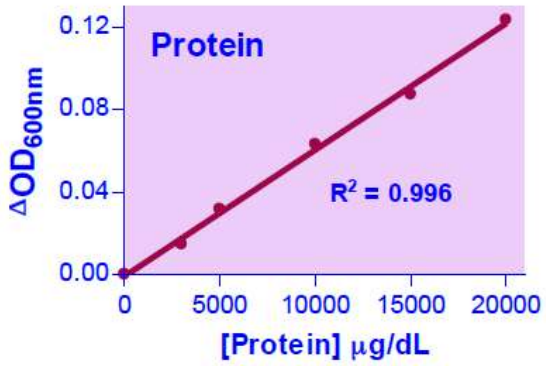
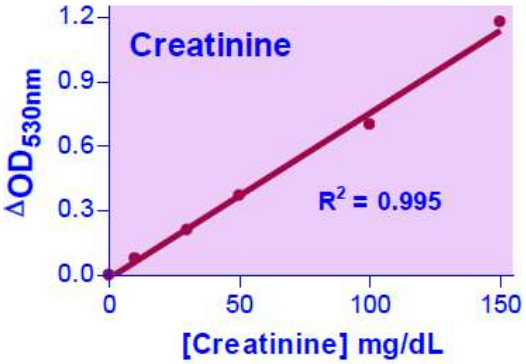


Figure 2.
Typical Creatinine Standard Curve



References

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2. Joern, W.A., and Schmoele, L., Urinary protein measurement by the Coomassie blue dye-binding method adapted to the ABA-l00 bichromatic analyzer. *Clin. Chem.*, **27**, 1305 (1981).
3. Toora, B.D., et al., Measurement of creatinine by Jaffe's reaction. *Indian J. Exp. Biol.*, **40(3)**, 352-4 (2002).

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