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ab272539 Protein Creatinine Ratio Assay Kit

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www.abcam.com/ab272539

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For quantitative determination of Protein Creatine Ratio (nitrate/nitrite) and evaluation of drug effects on its metabolism.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

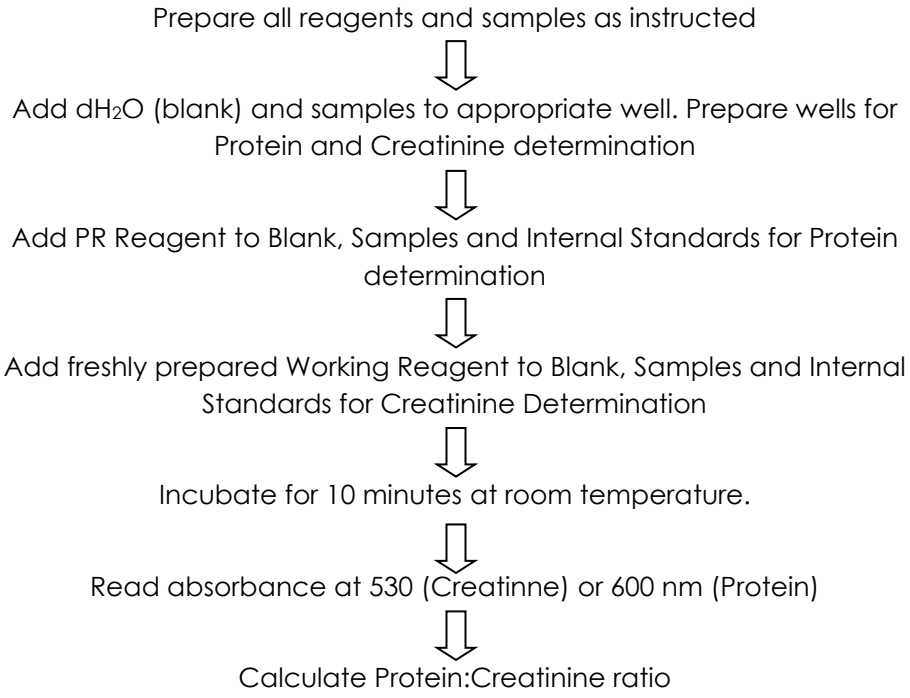
Protein Creatinine Ratio Assay Kit (ab272539) is a simple and convenient test for proteinuria. Other methods such as 24 hour urine test or timed urine test require strict adherence to sample collection protocol. Since the protein concentration is normalized to creatinine secretion, the urine sample can be taken at any time and no diet or liquid restrictions are necessary for sample collection.

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.

2. Protocol Summary



3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at 4°C immediately upon receipt. Kit has a storage time of 12 months from receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
CR Reagent A	6 mL	+4°C
CR Reagent B	6 mL	+4°C
PR Reagent	24 mL	+4°C
Standard	1 mL	+4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Distilled H_2O
- Pipettes
- 1.5 mL tubes
- Heat block or water bath
- Centrifuge
- 96-well clear plate with flat bottom
- Plate reader - capable of reading absorbance at 530 and 600 nm

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard and reagent.
- Pipette standards and samples to the bottom of the wells.
- Add the reagents to the side of the tube to avoid contamination.
- Some Solutions supplied in this kit are caustic; care should be taken with their use.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use.
- The kit contains enough reagents for 100 assays.

All reagents are supplied ready to use.

10. Standard Preparation

- Equilibrate standard to room temperature (18-25°C) prior to use.

Standard is supplied ready to use.

The assay protocol uses an internal standard rather than a standard curve.

11. Sample Preparation

Δ Note: 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.

Sample treatment:

11.1.1 If particulates are present, centrifuge samples.
11.1.2 Use the clear supernatant for assay.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

12.1 Protein Assay:

12.1.1 Add 20 µL of each sample into 4 separate wells: 2 Sample wells and 2 Internal Standard wells.
12.1.2 Add 5 µL dH_2O to Sample wells.
12.1.3 Add 5 µL of Standard to Internal Standard wells.
12.1.4 Add 25 µL of dH_2O into 2 wells (Blank).

ΔNote: Each sample does not require a separate Blank. The same Blank value can be used for all samples on a particular plate.

12.1.5 Add 200 µL of PR Reagent to each well.
12.1.6 Incubate 10 min at room temperature.
12.1.7 Read the optical density at 600 nm.

ΔNote: If the $OD_{STANDARD}$ - OD_{SAMPLE} for a particular sample is lower than 0.05, dilute sample with an equal volume of dH_2O 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.

12.2 Creatine Assay:

12.2.1 Prepare enough Creatine Working Reagent for all controls, samples and standards by mixing per well: 50 µL CR A, 50 µL CR Reagent B and 150 µL dH_2O.

Component	Working Reagent (µL/reaction)
Creatine Reagent A	50
Creatine Reagent B	50
dH_2O	150

ΔNote: Working Reagent is stable for 2 hours, we recommend making fresh reagents for each assay run.

12.2.2 Add 20 µL of each sample into 4 separate wells: 2 Sample wells and 2 Internal Standard wells.
12.2.3 Add 5 µL dH_2O to Sample wells.
12.2.4 Add 5 µL of Standard to Internal Standard wells.
12.2.5 Add 25 µL of dH_2O into 2 wells (Blank).

ΔNote: Each sample does not require a separate Blank. The same Blank value can be used for all samples on a particular plate.

12.2.6 Add 200 µL of Creatine Working Reagent to each well.
12.2.7 Incubate 10 min at room temperature.
12.2.8 Read the optical density at 530 nm.

ΔNote: If the $OD_{STANDARD}$ - OD_{SAMPLE} for a particular sample is lower than 0.1, dilute sample with an equal volume of dH_2O 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.

ΔNote: Use 96-well clear, flat-bottom plates.

13. Calculations

Protein concentration:

13.1.1 The Protein concentration of a Sample is calculated as follows:

$$[Protein] = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Sample}} X\ 10000\ X\ n\ (\mu g/dL)$$

OD_{Sample} = OD value of the sample
OD_{Blank} = OD value of water
$OD_{Standard}$ = OD value of standard

- 10,000 µg/dL is the concentration of the Protein Standard
- *n* is the dilution factor.

Creatine concentration:

13.1.2 The Creatine concentration of a Sample is calculated as follows:

$$[Creatine] = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Sample}} X\ 25\ X\ n\ (mg/dL)$$

OD_{Sample} = OD value of the sample
OD_{Blank} = OD value of water
$OD_{Standard}$ = OD value of standard

- 25 mg/dL is the concentration of the Creatine Standard
- *n* is the dilution factor.

Protein Creatine Ratio:

13.1.3 Protein Creatine Ratio of a Sample is calculated as follows:

$$[Protein\ Creatine\ Ratio] = \frac{[Protein]}{[Creatine]}\ (\mu g/mg)$$

Δ Note: 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).

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

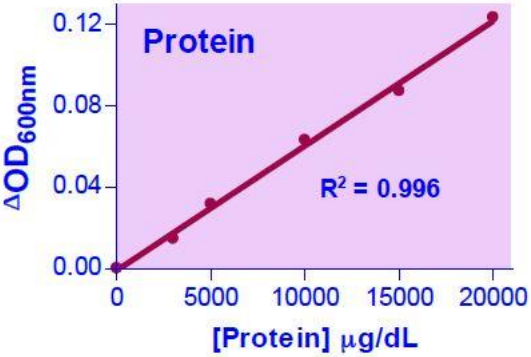


Figure 1. Example of Protein standard curve.

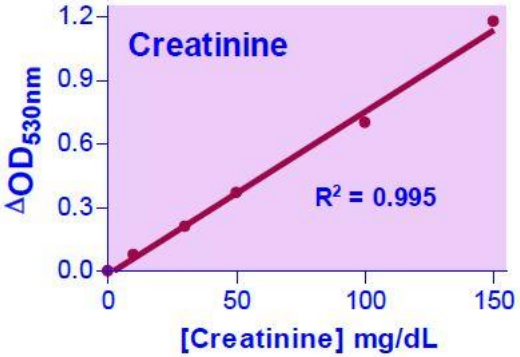


Figure 1. Example of Creatinine standard curve.

15. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/en-us/contact-us

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)