Rat Urinary Protein Assay Kit

Catalog # 9040

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	Assay kit to evaluate urinary protein concentration
FORMAT:	96-well ELISA Plate with removeable strips
ASSAY TYPE:	Turbidity Assay
ASSAY TIME:	10 minutes
STANDARD RANGE:	4 mg/ml to 0.4 mg/ml
NUMBER OF SAMPLES:	Up to 16 (duplicate) samples/plate
SAMPLE TYPES:	Urine
RECOMMENDED SAMPLE DILUTIONS:	1:2 (at least)
CHROMOGEN:	N/A (read at 450 nm)
STORAGE:	-20°C for 12 months
VALIDATION DATA:	N/A
NOTES:	N/A

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INTRODUCTION

The turbidity assay method (1) has been widely used to determine urinary protein levels in human specimens because it is accurate, easy, and economical. However, urine volume collected from rats varies from 0.1 ml to 20 ml during a 16-hour collection period, thus the volume is occasionally insufficient for current assay methods. In addition, turbidity (OD 450 nm) readings of individual test tubes using a spectrophotometer may be cumbersome. Chondrex, Inc.'s Rat Urinary Protein Assay Kit (Cat # 9040) uses the turbidity method in 96-well plates, offering a solution for assaying a large number of rat urine samples.

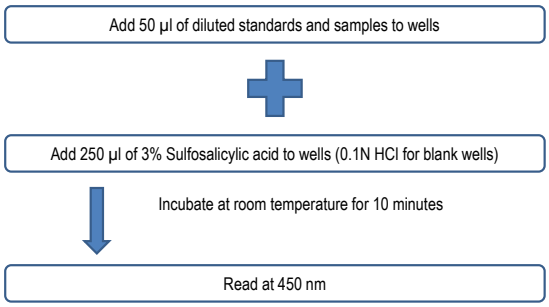
Protein concentrations in urine samples can be determined by turbidity or Bradford assay methods (2). The turbidity assay method which utilizes 3% sulfosalicylic acid is more convenient than the Bradford assay method for assaying a large number of samples because of the wide range of the dose response curve (Ex: 0.4 to 4 mg/ml) and the stable turbidity. Regardless of the assay method used, bovine serum albumin (BSA) cannot be used as a standard. For example, in the turbidity assay, the dose response curve generated by BSA significantly differs from that of serum proteins. In the Bradford assay, OD value of globulins is only 70% that of BSA (3). Therefore, a standard protein solution prepared from normal rat serum is ideal for assaying urinary protein levels instead of using BSA.

NOTE: The Bradford assay method requires two separate regression curves for assaying protein concentration, from 0.05 to 0.6 mg/ml and from 0.5 to 1.5 mg/ml. Because the protein concentration in rat urine can vary from 0.1 mg/ml to 50 mg/ml depending upon the progress of nephritis, various dilutions of individual samples are required.

KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard Protein Solution (90401)	1 vial	1.5 ml, 4 mg/ml	–20°C
PBS (90402)	1 bottle	30 ml	Room Temperature
0.1N HCl (90403)	1 bottle	40 ml	Room Temperature
3% Sulfosalicylic Acid (90404)	1 bottle	40 ml	Room Temperature
96-well Plate	2 plates	96-well (8-well strips x 12)	Room Temperature

ASSAY OUTLINE



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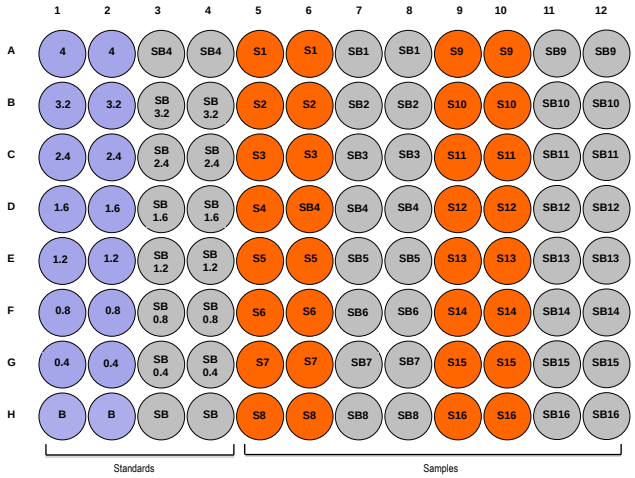
URINE COLLECTION PROTOCOL

Collect urine from 5 pm to 9 am every other day with metabolic cages. Measure the urine volume and centrifuge to remove insoluble materials. Keep the supernatant in a refrigerator for short-term storage and at –20°C for long-term storage.

PLATE LAYOUT

The assay should be performed in duplicate for both standards and samples. Because urine samples and even the standard may be colored due to hemoglobin contamination, it is important to determine the individual background values and subtract them from the turbidity values in sample wells. Therefore, columns 3-4, 7-8, and 11-12 are used as blank wells.



NOTES BEFORE USING ASSAY

NOTE 1: It is recommended that the standard and samples be run in duplicate.

NOTE 2: This kit contains animal components from non-infectious animals and should be treated as potential biohazards in use and for disposal.

ASSAY PROCEDURE

1. **Prepare Standards**: Add 0, 5, 10, 15, 20, 30, 40, and 50 µl of Standard Protein Solution (4 mg/ml) in duplicate into columns 1-2 (standard wells) and 3-4 (standard blank wells) from rows A to H according to the plate mapping layout. Add PBS to individual wells to adjust the final volume to 50 µl.

Standard (µl/well)	0	5	10	15	20	30	40	50
PBS (µl/well)	50	45	40	35	30	20	10	0
Total Volume (µl)	50	50	50	50	50	50	50	50
Final Concentration (mg/ml)	0	0.4	0.8	1.2	1.6	2.4	3.2	4.0

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2. **Prepare Samples**: Centrifuge urine samples at 10,000 rpm for 3 minutes using a tabletop microcentrifuge. Add 1-50 µl of urine supernatant in duplicate into columns 5-6 (test samples) and 7-8 (sample blanks) from rows A-H and into columns 9-10 (test samples) and 11-12 (blanks) from rows A-H according to the plate mapping layout. Add PBS to adjust the sample volume to 50 µl. Alternatively, dilute samples 1:2-1:50 or more with PBS and add 50 µl of diluted sample per well.

3. **Add Reagents**: Add 250 µl of 3% sulfosalicylic acid into standard and sample columns 1-2, 5-6, and 9-10, and 250 µl of 0.1N HCl into blank columns 3-4, 7-8, and 11-12. Incubate the plate for 10 minutes at room temperature and then read plate using a plate reader at OD 450 nm (single beam).

CALCULATING RESULTS

1. Average the duplicate OD values for the blank, standards, test samples, and sample blanks.

2. Subtract the averaged blank OD value from the individual averaged standard, standard blank, sample, and sample blank OD values. Subtract the averaged standard blank and sample blank OD values from their corresponding averaged standard and test sample OD values.

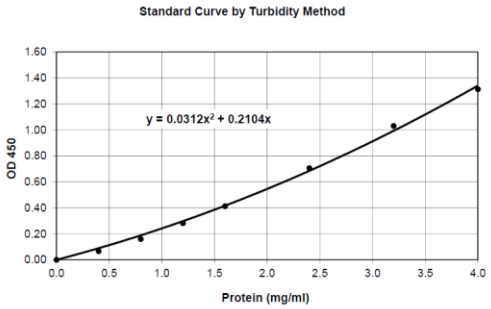
3. Plot the adjusted OD values of the standards against the concentration (mg/ml) of standard protein. Figure 1 shows representative OD values of the standard range from 0.4 - 4 mg/ml.

4. The protein levels in samples can be calculated using regression analysis. If necessary, multiply it by the sample dilution factor to obtain the urinary protein concentration in the original sample. For additional assistance, please download a sample calculation worksheet from www.chondrex.com. Copy the raw data into the template's cells C9-N16, redo the trendline and display the new trendline equation. Then type the "a" and "b" values from the equation of the standard regression curve into cells M38 and M39 respectively. Type in the volumes used for the samples (cells D60-D75) and PBS (cells E60-E75) to calculate sample dilution. The protein concentration in urine will be displayed in the last column (cells N60-N75).

   Determining the total amount of protein secreted in urine is preferred over the urinary protein concentration alone because the protein concentration is generally high when urine volume is small. Chondrex, Inc. recommends determining the total amount of protein secreted in urine in 16- or 24-hour periods to determine renal function.

   Total urinary protein (mg) = concentration (mg/ml) x volume of urine (ml)

Figure 1 - A Typical Standard Curve for the Rat Urinary Protein Assay Kit



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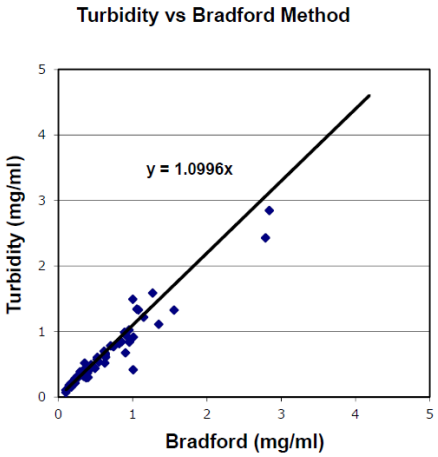
VALIDATION DATA

Seventy-two urine samples collected from normal and nephritic rats were assayed for their protein concentration using both the turbidity and the Bradford assay methods. Using an identical standard prepared from normal rat serum, the resulting data was analyzed for correlation by regression analysis. As shown in Figure 2, the protein concentration determined by both methods correlated well with a correlation coefficient of $r^2 = 0.928$.

Figure 2 - Correlation of Urinary Protein Levels Determined by the Turbidity and Bradford Assay Methods. For assaying protein concentration with the turbidity method, 50 µl of urine from normal rats and 10 µl from nephritic rats were used. Similarly, 10 µl from normal rats and 2.5 µl from nephritic rats were used for the Bradford method. In the Bradford method, 13 out of 72 samples were outside of the assay range due to high protein concentration. However, using the turbidity method, only 3 samples were out of assay range and needed to be re-assayed with further dilution.



TROUBLESHOOTING

For frequently asked questions about assays and ELISAs, please see Chondrex, Inc.'s ELISA FAQ for more information.

REFERENCES

1. H. Nishi, R. Elin, Three turbidimetric methods for determining total protein compared. *Clin Chem* **31**, 1377-80 (1985).

2. M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**, 248-54 (1976).

3. Bio-Rad Protein Assay. Bio-Rad Laboratories, California, USA

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