Rat Urinary Protein Assay Kit

Catalog # 9040

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INTRODUCTION

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, thus the volume is occasionally not enough for assaying proteins by current methods. In addition, turbidity (OD 450 nm) readings of individual test tubes using a spectrophotometer may be cumbersome. Chondrex's rat urinary protein assay kit, using a turbidity method in 96-well C-bottom microtiter plates, offers a solution for assaying a large number of rat urine samples and for calculating results automatically.

Protein concentrations in urine samples can be determined by both turbidity and Bradford assay methods (2). However, turbidity assay method using 3% sulfosalicylic acid is more convenient than the Bradford assay method for assaying urine protein concentration in a large number of samples, because of the wide range of dose response curve (0.4-4 mg/mL - Figure 1) and the turbidity stability for hours. Importantly, regardless of which assay method is used, bovine serum albumin (BSA) cannot be used as a standard. For example, the dose response curve of BSA significantly differs from the dose response curve of serum proteins using turbidity assay, and the OD value of globulins by Bradford assay is only 70% of the value of BSA (3). Therefore, a standard protein solution prepared from normal rat serum is ideal for assaying urinary protein levels instead of using BSA regardless of the assay method.

Note: Bradford assay method requires two separate regression curves for assaying protein concentration, from 0.05-0.6 mg/mL and from 0.5-1.5 mg/mL. Since the protein concentration in rat urine varies 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	Description	Quantity	Amount	Storage
Standard Protein Solution	Prepared from normal rat sera and can be used as a standard for rat urinary protein assay by both turbidity and Bradford assay methods	1 vial	1.5 mL, 4 mg/mL in PBS	-20°C
3% Sulfosalicylic Acid	Used for creating turbidity	1 bottle	40 mL	RT
0.1N HCl	Used for assaying blank values of standard and individual samples	1 bottle	40 mL	RT
PBS	Used for diluting samples	1 bottle	30 mL	RT
Microtiter Plates	96-well C-bottom plates for assay	2 each	96-well (12 x 8-well strips)	RT

NOTES BEFORE USING ASSAY

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

Note 2: There are enough reagents for two separate assays. Additional Standard Protein Solution (Catalog # 90401) may be purchased to run more assays. A total of 32 duplicate samples can be measured with this kit.

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URINE COLLECTION

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 –20°C for long-term storage.

ASSAY PROTOCOL BY TURBIDITY METHOD

1. **Mapping 96-Well C-Bottom Microtiter Plate**: Assay will be performed in duplicate for both standards and samples (see spreadsheet on page 3). Since urine samples and even the standard may have slight color due to contamination with hemoglobin, it is important to determine the individual background values and subtract them from the turbidity values in sample wells. Therefore, columns 1-2, 5-6 and 9-10 (B1 and B2) are used as blank wells.

2. **Standard Preparation**: Add 0, 5, 10, 15, 20, 30, 40 and 50 μL of Standard Protein Solution (4 mg/mL) in duplicate into columns 1-2 (blank wells) and 3-4 (standard wells) from rows A to H. 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	3.6	4.0

3. **Urine Sample Preparation**: Centrifuge urine samples at 10,000 rpm for 3 minutes using a tabletop micro-centrifuge. Add 1-50 μL of urine supernatant in duplicate into columns 5-6 (blank) and 7-8 (test) from rows A-H, and into columns 9-10 (blank) and 11-12 (test) from rows A-H of the 96-well microtiter plate. Add PBS to adjust the sample volume to 50 μL. Alternatively, dilute samples 1:2-1:50 or more, if necessary, with PBS. Add 50 μL of diluted sample per well.

4. **Turbidity Assay**: Add 250 μL of 0.1N HCl into blank columns 1-2, 5-6 and 9-10, and 250 μL of 3% sulfosalicylic acid into columns 3-4, 7-8 and 11-12. Allow the microplate to stand for 10 minutes at room temperature, and read plates using an ELISA reader with single beam at OD 450 nm.

5. **Calculating Protein Concentration**: Copy the obtained data into Excel spreadsheet (www.chondrex.com under product page for Rat Urinary Protein Assay Kit in the Nephritis section) cells C9-N16, redo trendline and display new trendline equation, then type the "a" and "b" values from the equation of standard regression curve into cells M60 and M61 respectively. Type the volumes of sample (cells D40-D55) and PBS (cells E40-E55) added to the wells to calculate sample dilution. The protein concentration in urine will be displayed in the last column (cells N40-N55).

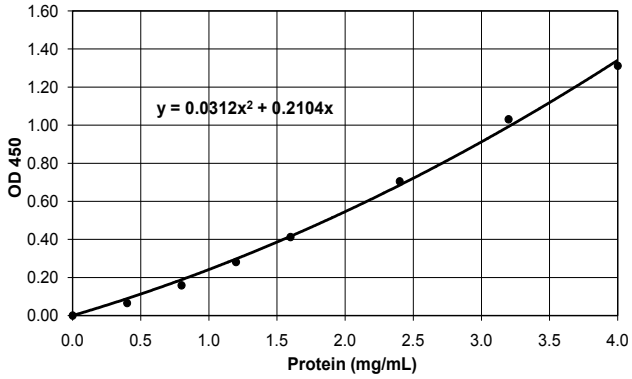

Figure 1 - A typical standard curve for rat urinary protein assay

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Turbidity Assay for Rat Urinary Protein

Mapping

Copy the data from ELISA reader into the table below with red.

Standard (0-4 mg/mL)				Test Sample (1-8)				Test Sample (9-16)			
1	2	3	4	5	6	7	8	9	10	11	12
B1	B2	S1	S2	B1	B2	T1	T2	B1	B2	T1	T2
0.033	0.031	0.032	0.032	0.054	0.056	1.365	1.365	0.054	0.056	1.365	1.365
0.041	0.038	0.107	0.105	0.054	0.056	1.365	1.365	0.054	0.056	1.365	1.365
0.041	0.042	0.198	0.203	0.054	0.056	1.365	1.365	0.054	0.056	1.365	1.365
0.041	0.042	0.328	0.319	0.054	0.056	1.365	1.365	0.054	0.056	1.365	1.365
0.048	0.050	0.462	0.461	0.054	0.056	1.365	1.365	0.054	0.056	1.365	1.365
0.049	0.052	0.751	0.760	0.054	0.056	1.365	1.365	0.054	0.056	1.365	1.365
0.055	0.055	1.082	1.089	0.054	0.056	1.365	1.365	0.054	0.056	1.365	1.365
0.054	0.056	1.365	1.370	0.054	0.056	1.365	1.365	0.054	0.056	1.365	1.365

B1-B2 - Blank Wells, S1-S2 - Standard Wells, T1-T2 - Test Sample Wells

Add 0.1N HCl to blank wells in yellow and 3% sulfosalicylic acid to standard and test wells in pink.

Standard Curve

Obtain "a" and "b" values from the graph below, and type them in the cells next to "a" and "b", respectively.

Protein (mg/mL)	Standard 4 mg/mL (µL)	PBS (µL)	0.1N HCl or Sulfosalicylic Acid* (µL)	Total Volume (µL)	OD 450 (Blank) B1	OD 450 (Blank) B2	OD 450 (Test Sample)* S1	OD 450 (Test Sample)* S2	OD450 (Corrected)	Standard Protein (mg/mL)
0.00	0	50	250	300	0.033	0.031	0.032	0.032	0.000	0.00
0.40	5	45	250	300	0.041	0.038	0.107	0.105	0.067	0.30
0.80	10	40	250	300	0.041	0.042	0.198	0.203	0.159	0.69
1.20	15	35	250	300	0.041	0.042	0.328	0.319	0.282	1.15
1.60	20	30	250	300	0.048	0.050	0.462	0.461	0.413	1.59
2.40	30	20	250	300	0.049	0.052	0.751	0.760	0.705	2.46
3.20	40	10	250	300	0.055	0.055	1.082	1.089	1.031	3.29
4.00	50	0	250	300	0.054	0.056	1.365	1.370	1.313	3.94

Note* - Add 3% sulfosalicylic acid in test sample wells instead of 0.1N HCl.

Assay Results

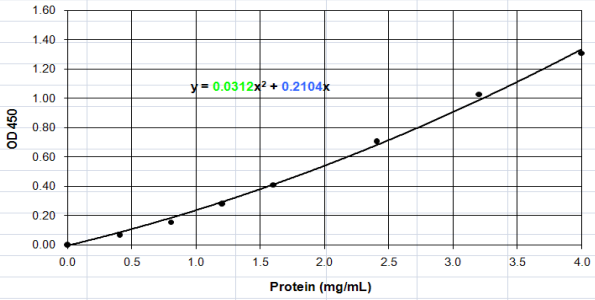
Type the sample and PBS volumes in the table below.

Test Sample No.	Sample (µL)	PBS (µL)	Dilution (x)	0.1N HCl or Sulfosalicylic Acid* (µL)	Total Volume (µL)	OD 450 (Blank) B1	OD 450 (Blank) B2	OD 450 (Test Sample)* T1	OD 450 (Test Sample)* T2	OD450 (Corrected)	Sample Protein (mg/mL)
1	5	45	10	250	300	0.054	0.056	1.365	1.365	1.310	39.33
2	5	45	10	250	300	0.054	0.056	1.365	1.365	1.310	39.33
3	10	40	5	250	300	0.054	0.056	1.365	1.365	1.310	19.66
4	10	40	5	250	300	0.054	0.056	1.365	1.365	1.310	19.66
5	10	40	5	250	300	0.054	0.056	1.365	1.365	1.310	19.66
6	10	40	5	250	300	0.054	0.056	1.365	1.365	1.310	19.66
7	10	40	5	250	300	0.054	0.056	1.365	1.365	1.310	19.66
8	10	40	5	250	300	0.054	0.056	1.365	1.365	1.310	19.66
9	10	40	5	250	300	0.054	0.056	1.365	1.365	1.310	19.66
10	10	40	5	250	300	0.054	0.056	1.365	1.365	1.310	19.66
11	10	40	5	250	300	0.054	0.056	1.365	1.365	1.310	19.66
12	10	40	5	250	300	0.054	0.056	1.365	1.365	1.310	19.66
13	10	40	5	250	300	0.054	0.056	1.365	1.365	1.310	19.66
14	10	40	5	250	300	0.054	0.056	1.365	1.365	1.310	19.66
15	50	0	1	250	300	0.054	0.056	1.365	1.365	1.310	3.93
16	50	0	1	250	300	0.054	0.056	1.365	1.365	1.310	3.93

Note* - Add 3% sulfosalicylic acid in test sample wells instead of 0.1N HCl.

Standard Curve by Turbidity Method

a= 0.0312
b= 0.2104



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DATA ANALYSIS

Total amounts of proteins secreted into urine reflect the renal function rather than the urinary protein concentration, because the protein concentration is generally significantly higher when urine volume is small. We recommend using total amounts of proteins secreted into urine during 16 or 24 hours for determining renal function.

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

CORRELATION OF PROTEIN LEVELS DETERMINED BY TURBIDITY AND BRADFORD ASSAY METHODS

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

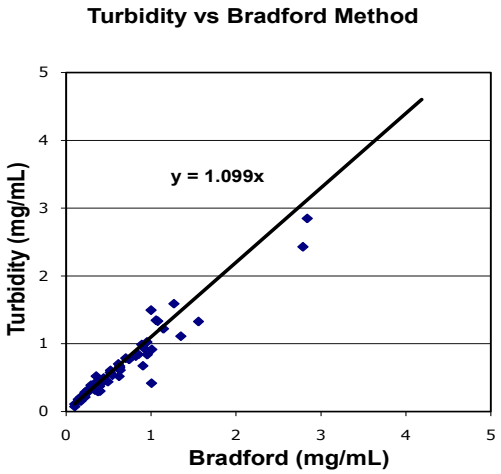

Figure 2 - Correlation of urinary protein levels determined by turbidity and Bradford assay methods. For assaying protein concentration by turbidity method, 50 μL of urine from normal and 10 μL from nephritis rats were used. Similarly, 10 μL from normal rats and 2.5 μL from nephritis rats were used for assay by Bradford method. Thirteen out of 71 samples were out of the assay range due to the high protein concentration by Bradford method, whereas only three samples needed to be re-assayed after further dilution by turbidity method, and were excluded from this graph.

REFERENCES

1. Nishi HH and Elin RJ. Three turbidimetric methods for determining total protein compared. Clin. Chem. 31: 1377-80 (1985)

2. Bradford M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254 (1976)

3. BioRad protein assay. Bio-Rad Laboratories, California, USA

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