Rat Urinary Protein Assay Kit

Catalog # 9040

For Research Use Only - Not Human or Therapeutic Use

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 utilizies 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 (0.4 to 4 mg/ml - Figure 1) 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% of 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	RT
0.1N HCl (90403)	1 bottle	40 ml	RT
3% Sulfosalicylic Acid (90404)	1 bottle	40 ml	RT
96-well Plate	2 plates	96-well (8-well strips x 12)	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 at –20°C for long-term storage.

ASSAY PROTOCOL

1. **Mapping 96-Well Plate**: The assay will be performed in duplicate for both standards and samples (see spreadsheet on page 3). 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 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	3.2	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 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. Incubate the plates for 10 minutes at room temperature and then read plates using a plate reader at OD 450 nm (single beam).

5. **Calculating Protein Concentration**: Copy the obtained data into the provided Excel spreadsheet (www.chondrex.com under the product page for the Rat Urinary Protein Assay Kit) cells C9-N16, redo the trendline and display the new trendline equation. Then type the "a" and "b" values from the equation of 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).

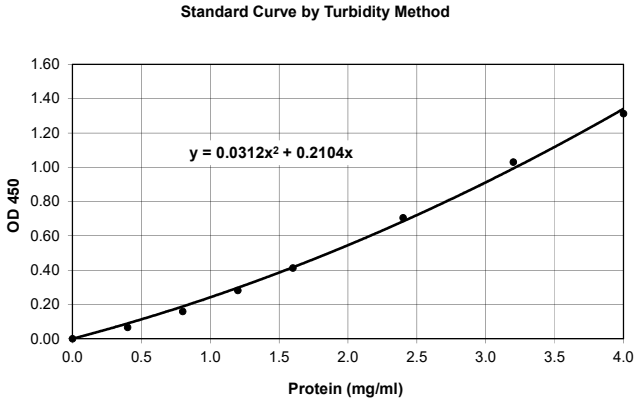

Figure 1 - A typical standard curve for rat urinary protein assay

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Turbidity Assay for Rat Urinary Protein

Mapping Copy the data from the ELISA reader into the table, replacing the values below in 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.

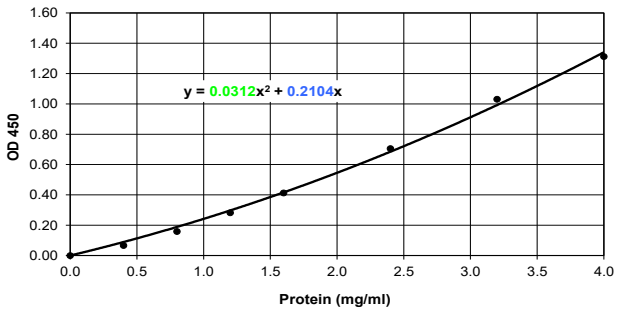
Standard Curve Obtain "a" and "b" values from the graph below, and type them in the cells next to "a" and "b", respectively.

Protein (mg/ml)	Standard 4 mg/µl (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.

Standard Curve by Turbidity Method

a= 0.0312
b= 0.2104



Assay Results Copy sample and PBS values into the table, replacing the red values 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.

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DATA ANALYSIS

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 during 16 or 24 hours to determine 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 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 $r^2 = 0.928$.

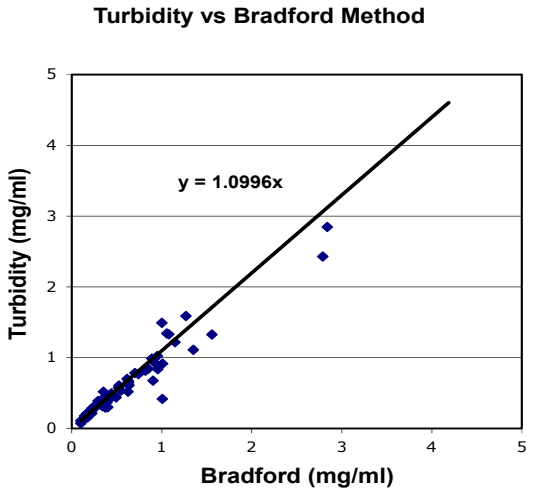

Figure 2 - Correlation of urinary protein levels determined by the turbidity and Bradford assay methods. For assaying protein concentration with the turbidity method, 50 μl of urine from normal rats and 10 μl from nephritic rats were used. Similarly, 10 μl from normal rats and 2.5 μl from nephritic rats were used for the Bradford method. In the Bradford method, 13 out of 72 samples were outside of the assay range due to the 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.

REFERENCES

1. H. Nishi and 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**: 248-254 (1976)

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

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