

Kit for measurement of Rat Albumin according to EIA method
PRE061

Pana-test Rat Albumin

1. Introduction

Since urinary albumin increase is associated with release of serum protein due to glomerular basement membrane lesions, this becomes the indication for glomerular inflammation, renal amyloidosis and diabetic glomerular nephropathy. This reagent can detect micro doses of albumin, which can not be detected by qualitative testing paper for urinary protein, with high sensitivity by EIA method which incorporates specific antibody to rat albumin.

2. Characteristics

1. Since this includes an exclusive reagent for quantitative determination in rats, specific and precise data can be obtained.
2. No special facility is necessary, due to EIA method.

3. Composition of the reagent kit

1. ELISA plate (Anti-rat albumin antibody-coated solid phase plate) 96wells \times 1
2. Standard rat albumin ($2\mu\text{g/mL}$) for 1mL (lyophilized) \times 1
3. Concentrated sample diluent buffer (5-fold concentrated, for 200mL use) 40mL \times 1
4. Enzyme - labeled antigen (Peroxidase-conjugated rat albumin) for 6mL use (lyophilized) \times 1
5. Chromogenic tablet (Containing 10mg of *o*-phenylenediamine in a tablet) for 8mL/tablet \times 2
6. Substrate solution (Containing 4.0mg of hydrogen peroxide) 20mL \times 1
7. Concentrated washing solution (10-fold concentrated PBS-Tween 20, for 400 mL use) 40mL \times 1
8. Stopping solution (2N sulfuric acid) 15mL \times 1

4. Method for preparation of test reagents.....

Test reagent	Method for preparation	Reagent prepared	Method and terms for valid storage
① ELISA plate	Remove from aluminum package and wait until plate reaches room temperature. Put 300 μ L of washing solution into each of 96 wells just before use, and leave them standing for 10 minutes.	Plate for changing into solid phase	Prepare wells in a necessary number, freshly before use.
② Standard rat albumin	Accurately add 1.0mL of purified water*. Thoroughly mix it for complete dissolution, with care not to form any bubbles.	Standard rat albumin, 2 μ g/mL	Solution is stable for one week when stored in the dark and cool place (2-10°C).
③ Concentrated sample diluent buffer	Add the whole volume of 40mL into 160mL of purified water*, and mix it thoroughly.	Sample diluent buffer	Stable in the dark and cool place (2-10°C).
④ Enzyme-labeled antigen	Accurately add 6mL of purified water* to vial, and mix it thoroughly.	Enzyme-labeled antigen solution	Antigen solution is stable for one week in the dark and cool place (2-10°C).
⑤ Chromogenic tablet ⑥ Substrate solution	Collect 8mL of the substrate solution. Add it to one chromogenic tablet. Thoroughly mix for complete dissolution.	Chromogenic substrate solution	Freshly prepare it, just before use.
⑦ Concentrated washing solution	Add the whole volume of 40mL into 360mL of purified water*, and thoroughly mix it.	Washing solution (PBS-0.05% Tween 20)	Washing solution is stable for one month at room temperature.
⑧ Stopping solution	Use it in its intact form.		Stable at room temperature.

.....Important notes.....

* : Distilled or deionized water

Make sure that all the test reagents are used after returning to room temperature.

Wells not used out of those of the ELISA plate must be kept tightly-sealed in the aluminum package in which the plate was kept, and must be kept in the dark and cool place.

The chromogenic substrate should not be stored for prolonged period after mixing ⑤ with ⑥.

5. Necessary instrument and apparatuses

1. Micro pipette and tips ($50\ \mu\text{L}$, $100\ \mu\text{L}$ - $1000\ \mu\text{L}$)
2. Mass pipette (1mL,10mL)
3. Mass cylinder (500mL)
4. Cleaning instrument for 96 wells microtiter plate
(In the case of manual operation: Continuous distributor, aspirator, etc.)
5. Multi-channel pipette
6. Microtiter plate reader (With measuring wave length of 492nm)

6. Operation method for measurement

Preparation of standard rat albumin solution

Accurately add 1.0mL of purified water into the vial containing the ② standard rat albumin, providing the concentration of $2\ \mu\text{g/mL}$. Dilute the original solution in a series so as to prepare varying dilutions of 1000ng/mL , 500ng/mL , 250ng/mL , 125ng/mL , 63ng/mL , 31ng/mL , and 16ng/mL . For 0ng/mL , use the test sample diluent buffer in its intact form.

Preparation of test samples

Keep the urine samples below -20°C . As approximately, $50\text{-}60\ \mu\text{g}$ of the albumin is typically contained in 1mL of urine, dilute the samples whenever assumed as necessary.

Example: Add $950\ \mu\text{L}$ of the ③ sample diluent buffer into $50\ \mu\text{L}$ of urine to make the mixture into 20-fold volume. Collect $50\ \mu\text{L}$ out of it and add $950\ \mu\text{L}$ of the ③ sample diluent buffer to it to make the mixture into 400-fold volume. (As carry-over of the test sample may be possible, it is recommended to replace the pipette tip for each dilution.) For the test samples in high concentrations which are assumed to be so high as to be out of the measurable range, dilute the test sample with the ③ sample diluent buffer.

The measurement is feasible also with the supernatant of culture containing 10% fetal calf serum. (The results of the dilution tests and the recovery tests after addition are good.)

As the amount of albumin in the cultured supernatant is assumed to vary with the test conditions, it is recommended to perform preparatory tests.

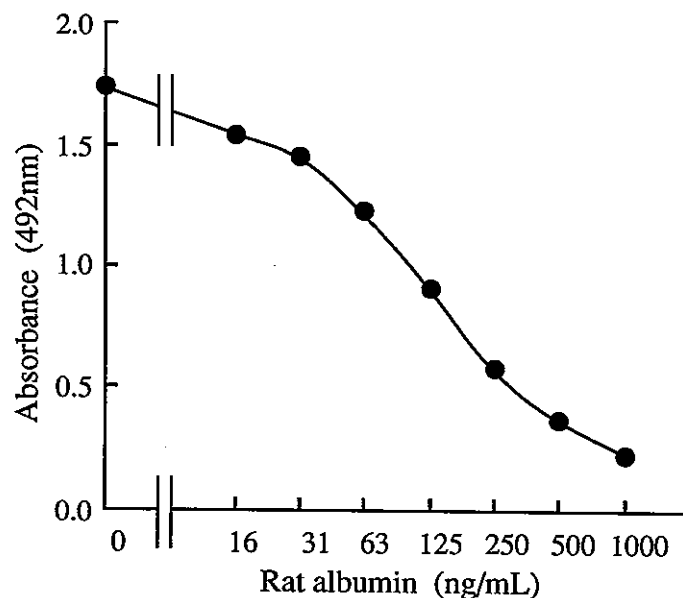
Procedures for measurement operation

It is recommended to conduct all the measurements in duplicity or in a higher multiplicity.

- 1) Take the ① ELISA plate out of the aluminum seal, and distribute 300 μ L each of the ⑦ washing solution into each well to be used. Leave them standing for 10 minutes at room temperature. (There is no adverse effect, even when it is left standing for up to 30 minutes.)
- 2) Remove the liquid in the well by suction with an aspirator.
- 3) Add 50 μ L each of the ② standard rat albumin in the concentrations of 0 - 1000ng/mL or the test sample into each well. Further add 50 μ L each of the ④ enzyme-labeled albumin into each well. Thoroughly mix it with a plate mixer and leave it standing for one hour at room temperature.
- 4) Remove the liquid in the well by suction with an aspirator, and distribute 300 μ L each of the ⑦ washing solution into each well. Next, remove the washing solution from each well.
- 5) Repeat the same step as in 4) twice, for further washing.
- 6) Add 100 μ L each of the ⑤, ⑥ chromogenic substrate solution, prepared as above, into each well in the designated order and at a fixed intervals, reacting at room temperature for 10 minutes.
- 7) Add 50 μ L each of the ⑧ stopping solution into each well in the same order and at the same interval as the addition of the chromogenic substrate solution, so as to stop the enzyme reaction.
- 8) Measure the absorbance at 492 nm with a microtiter plate reader.

7. Method for calculation of rat albumin concentration

- 1) Calculate the mean value of each absorbance obtained by the duplicate measurements.
- 2) Plot the concentration of the standard solution on the X-axis and the value of the absorbance on the Y-axis, to thus prepare the standard curve.
- 3) Apply the values of the absorbance of the test sample into the standard curve, so as to read the rat albumin concentration in the test sample, and multiply this concentration by the dilution multiple.



8. Precautions for the measurement

- 1) Strictly observe the term and the method for storage for each test reagent.
- 2) Make sure to return the prepared test reagents to room temperature before actual use.
- 3) Use each test reagent after confirming that each of them is completely dissolved.
- 4) Take care to not inflict damage to any well when removing the reaction solution from each well by suction.
- 5) For measurement of many test samples, take care that the reaction time of each test sample is at a fixed time as designated.
- 6) Prepare the standard curve freshly for each measurement.
- 7) Thoroughly clean the instrument for preparation of the chromogenic substrate solution before actual use.
(Color development may take place due to contamination of the instrument.)
- 8) White powder may sometimes be found, adhered to the wells of the ELISA plate. This is due to the dried blocking solution, but will not give any adverse effect upon the measurement.
- 9) As the ⑧ stopping solution is 2N sulfuric acid, take care when handling it.

9. Performance of the system

Range of measurement

Within the range of 16 - 1000ng/mL, rat albumin can be measured with this system.

Intra - assay precision

Standard		Mean value of absorbance	C.V.(%)
Rat albumin (ng/mL)			
0	(N=8)	1.740	2.2
16	(N=8)	1.538	2.2
31	(N=8)	1.449	2.5
63	(N=8)	1.231	0.7
125	(N=8)	0.909	1.4
250	(N=8)	0.584	3.2
500	(N=8)	0.370	6.2
1000	(N=8)	0.234	8.2

C.V. = Coefficient of variation

Test sample			
Urine		Mean value of absorbance	C.V.(%)
A	(N=8)	0.672	4.5
B	(N=8)	0.812	5.1
C	(N=8)	0.885	2.4
Urine		Mean value of concentration (ng/mL)	C.V.(%)
A	(N=8)	218	6.8
B	(N=8)	162	9.2
C	(N=8)	139	4.5

The urine samples A, B and C are the test samples of urine of SD rats (at age of 7 weeks, male), with dilution into 400-fold volume.

Inter - assay precision

Standard			
Rat albumin (ng/mL)		Mean value of absorbance	C.V.(%)
0	(N=8)	1.733	3.6
16	(N=8)	1.563	2.7
31	(N=8)	1.453	3.2
63	(N=8)	1.266	5.2
125	(N=8)	0.991	6.9
250	(N=8)	0.687	8.9
500	(N=8)	0.420	8.4
1000	(N=8)	0.265	8.0

Test sample			
Urine		Mean value of absorbance	C.V.(%)
A	(N=8)	0.782	8.5
B	(N=8)	0.916	6.7
C	(N=8)	1.011	6.5
Urine		Mean value of concentration(ng/mL)	C.V.(%)
A	(N=8)	203	6.8
B	(N=8)	150	5.2
C	(N=8)	120	10.2

The urine samples A, B and C are the test samples of urine of SD rats (at age of 7 weeks, male), with dilution into 400-fold volume.

Test of recovery after addition

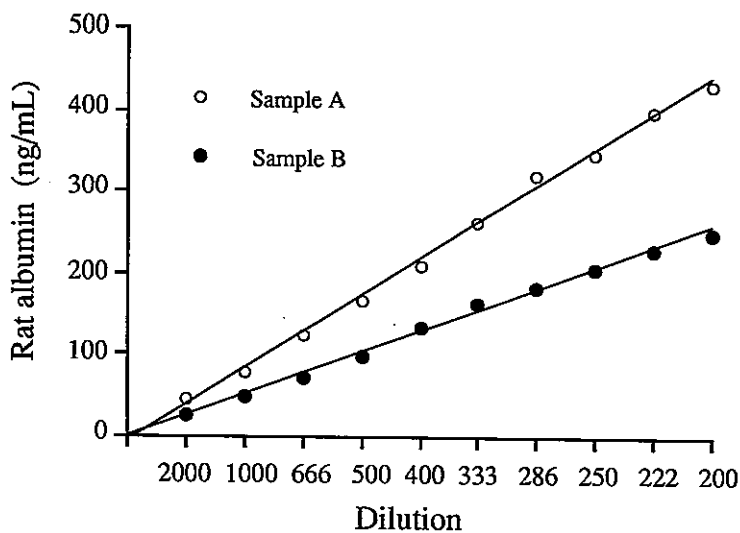
The results of measurement with urine samples of SD rats (male, at age of 7 weeks), with dilution into 400-fold volume to which the standard rat albumin was added were as shown below.

Test sample : Urine

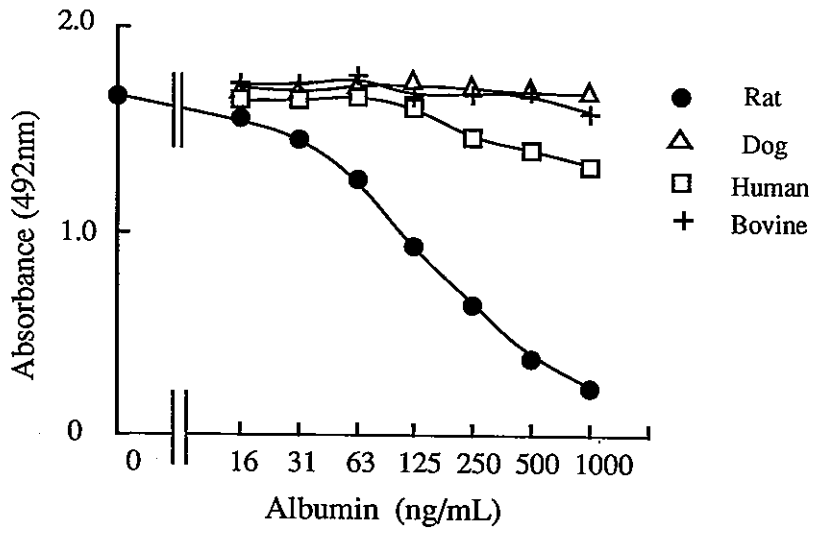
Amount of addition (ng/mL)	Value of actual measurement(ng/mL)	Theoretical value (ng/mL)	Recovery ratio (%)
0	48	-	-
50	110	98	112.2
100	153	148	103.4
200	251	248	101.2
400	417	448	93.1

Dilution test

Linearity with dilution can be obtained within the range of 200 - 2000 - fold dilution of urine samples of SD rats (at age of 7 weeks, male).

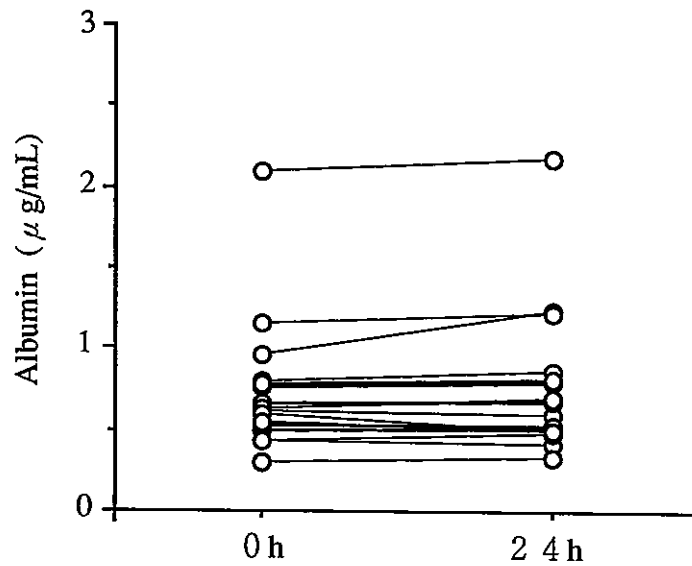


Specificity



Stability of test samples

Even after storage for 24 hours at 24°C of 19 test samples of urine of SD rats, hardly any changes were noted in the content of albumin in the urine.



10. Method for storage and terms of validity

Stability is assured until the demonstrated expiration date (one year after manufacture), following storage in the dark and cool place (2-10°C).

11. Package

96 units for test.