Reagent kit for measurement of Rat Rat Albumin
For research purpose only

Prepared in August 2008
PRD051

Pana-test Rat Albumin

1. Introduction

Increases of urinary albumin are associated with the leakage of plasma proteins into urine due to lesions of the glomerular basement membrane. This phenomenon can act as an indicator for kidney injury, such as glomerular inflammation, renal amyloidosis and diabetic glomerular nephropathy. This ELISA kit bearing a specific antibody to rat albumin, can detect low levels of albumin, which be never detected by using testing papers for urinary proteins.

2. Characteristics

- This kit includes an exclusive reagent for quantitative determination of rat albumin.
- No specific facility is necessary.

3. Components of the Kit

- ELISA plate (anti-rat Albumin antibody-coated microplate) ..... 1 plate
- Standard rat Albumin (2 μg/mL) for 1 mL (lyophilized)..... 1 vial
- Sample diluent concentrate, 40 mL (5-fold concentrated, for 200 mL use)..... 1 vial
- Enzyme-labeled antibody (peroxidase-conjugated anti-rat albumin antibody) for 6 mL (lyophilized)..... 1 vial
- Chromogen solution (containing 13.2 mg of 3,3',5,5'-tetramethylbenzidine in 0.5 mL of *N,N*-dimethylformamide)..... 1 vial
- Substrate solution, 20 mL (containing 0.0083 w/v% hydrogen peroxide)..... 1 vial
- Washing buffer concentrate, 40 mL (10-fold concentrated PBS-Tween 20, for 400 mL use)..... 1 vial
- Stop solution, 15 mL (1 mol/L sulfuric acid)..... 1 vial

4. Reagent Preparation

Component	Preparation	Reagent prepared	Storage condition and stability
① ELISA plate	Wait until the plate reaches to room temperature. Add 300 µL of wash buffer to each well just before use, and leave for 10 minutes.	Anti-rat albumin antibody-coated plate	Prepare a required number of strip only immediately before use.
② Standard rat albumin	Add exactly 1.0 mL of purified water[1)] to the vial, and mix it thoroughly for complete dissolution. Be careful not to form bubbles.	Standard rat albumin (2 µg/mL)	Stable in a refrigerator (2 to 10°C) for one week
③ Sample diluent concentrate	Add the entire volume of the concentrate (40 mL) into 160 mL of purified water, and mix it thoroughly.	Sample diluent	Stable in a refrigerator (2 to 10°C) for one week
④ Enzyme-labeled antibody	Add exactly 6 mL of purified water to the vial, and mix it thoroughly.	Enzyme-labeled antibody solution	Stable in a refrigerator (2 to 10°C) for one week
⑤ Chromogen solution ⑥ Substrate solution	Add 100 µL of the chromogen solution into 10 mL of the substrate solution.	Chromogenic substrate solution	Freshly prepare, just before use.
⑦ Wash buffer concentrate	Add the entire volume of the concentrate (40 mL) into 360 mL of purified water, and mix it thoroughly.	Wash buffer [2)]	Stable at room temperature for one week
⑧ Stop solution	Use it as it is		Stable at room temperature

1) Distilled or deionized water
2) PBS containing 0.05 v/v% Tween 20

NOTE: *: Distilled or deionized water
All reagents should be allowed to equilibrate to room temperature before use.
Disused strips should be closed up in a foil pouch and stored at 2 to 10°C in the dark.
Immediately use the chromogenic substrate solution after mixing ⑤ with ⑥.

5. Supplies Required

- Micropipettes and pipette tips (50 µL, 100 to 1,000 µL)
- Blowout pipettes (1 mL, 10 mL)
- Graduated cylinder (500 mL)
- Squirt bottle, manifold dispenser, or automated microplate washer
- Multi-channel pipette
- Microplate reader capable of measurement at or near 450 nm
- Distilled or deionized water

6. Assay Procedure

6.1 Preparation of Standard Rat Albumin Solutions

Reconstitute the standard rat albumin with accurately 1.0 mL of deionized or distilled water, producing a 2 μg/mL standard. Swirl or mix gently and leave for a while to ensure complete reconstitution. Make serial dilutions of the 2 μg/ mL standard with the sample diluent, to prepare the standard solutions at 1000, 500, 250, 125, 63, 31 and 16 ng/mL.
Distribute the sample diluent to the 0 ng/mL standard.

6.2 Sample Dilution

Urine sample

Store the samples below -20°C. As the content of albumin is deemed to be in the order of μg per mL of urine, appropriately dilute it prior to assay based on the expected concentration.

ex.) Add 950 μL of the sample diluent into 50 μL of urine (20-fold dilution), then add 950 μL of the sample diluent into 50 μL of the 20-fold diluted sample (final 400-fold dilution).
If you suspect the albumin concentration in a test sample exceeds the highest point (1,000 ng/mL) of the standard curve, we suggest that test sample should be diluted with the sample diluent. (As carry-over of the sample may be possible, it is recommended to replace a tip for each dilution.)

6.3 Assay Protocol

Bring all reagents and samples to room temperature before use. It is recommended that all samples, including the standards, are assayed in duplicate.

1) Add 300 μL of the wash buffer to each well of the ELISA plate. Incubate for 10 minutes at room temperature. (no adverse effect, even if left standing for up to 30 minutes.)
2) Aspirate each well to remove the solution.
3) Add 50 μL of the standard rat albumin or unknown samples to each well, and add 50 μL of the enzyme-labeled antibody solution to each well and mix, and incubate for 1 hours at room temperature.
4) Aspirate each well and wash the wells with wash buffer (300 μL/well). Repeat the washing procedure twice more. The complete removal of an aqueous fluid in each wash is essential for good performance.
5) Wash the wells as in step 4.
6) Add 100 μL of the chromogenic substrate solution to each well and incubate at room temperature for 15 minutes.
7) Add 50 μL of the stop solution to each well.
8) Measure an absorbance at 450 nm (A_{450}) with a microplate reader.

7. Data Calculation

1) Average the duplicate reading for each standard and each sample.
2) Plot the values of A_{450} (Y-axis) versus the concentrations of the standard solutions (X-axis), to draw a standard curve.

3) Apply an A_{450} value of each sample in the standard curve, so as to read a rat albumin concentration in the sample.
4) In case of a diluted, multiply the albumin concentration by the dilution factor to get the albumin concentration in the urine sample.

8. Safety Warnings and Precautions

- Strictly observe the storage condition for each reagent.
- All reagents should be brought to room temperature before use.
- Use reagents after confirming complete dissolution and uniformity.
- Take care not to inflict damage on any well when aspirating an aqueous fluid in each well.
- When measuring many samples in one assay batch, the time period of each reaction for all samples should be uniformed at a fixed time as designated.
- Prepare a standard curve for every measurement.
- Prepare the substrate solution with a clean vessel.
- White powder may sometimes be found in the wells of the ELISA plate. This is due to the dried blocking solution, but will have no effect on the measurement.
- Take care to handle the stop solution, it is very harmful.

9. Performance Characteristics

9.1 Quantitative Range

16 – 1,000 ng/mL of rat albumin

9.2 Intra - assay Precision

Standards

Rat albumin (ng/mL)	(Replicate)	A_{450} (mean)	C.V. (%)
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

Samples

Sample	(Replicate)	A_{450}		Albumin conc. (ng/mL)	
		mean	CV (%)	mean	CV (%)
A	(N=8)	0.672	4.5	218	6.8
B	(N=8)	0.812	5.1	162	9.2
C	(N=8)	0.885	2.4	139	4.5

C.V. = coefficient of variation

Sample A, B and C: 400-fold dilution of rat urine specimens (male, 7 weeks of age)

9.3 Inter-assay Precision

Standards

Rat albumin (ng/mL)	(Replicate)	A_{450} (mean)	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

Samples

Sample	(Replicate)	A_{450}		Albumin conc.	
		mean	CV (%)	mean	CV (%)
A	(N=8)	0.782	8.5	203	6.8
B	(N=8)	0.916	6.7	150	5.2
C	(N=8)	1.011	6.5	120	10.2

C.V. = coefficient of variation

Sample A,B and C: 400-fold dilutions of rat urine specimens (male, 7 weeks of age)

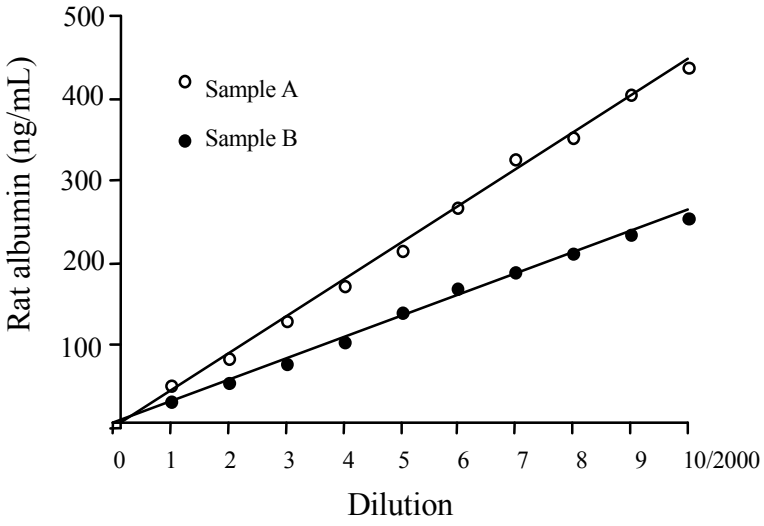
9.4 Recovery

Samples were prepared by spiking the four levels of standard rat albumin into rat urine (male, 7 weeks of age).

Sample	Spiked amount (ng/mL)	Measured value (ng/mL)	Expected value (ng/mL)	Recovery (%)
Urine	0	48	-	-
	50	110	98	112.2
	100	153	148	103.4
	200	251	248	101.2
	400	417	448	93.1

9.5 Linearity

Samples were prepared by a serial dilution of the rat urine samples with the sample diluent from 200- to 2,000-fold.



10. Storage and Expiry

Store all reagents at 2-10°C under dark and use until a stated expiration date (one year after manufactured).

11. Package

96 tests per kit

GENTAUR

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, BELGIUM
Tel 0032 16 58 90 45 | Fax 0032 16 50 90 45
www.gentaur-worldwide.com
info@gentaur.com