SHIBAYAGI	Rat Albumin ELISA Kit	Research Reagent

Please, read this instruction carefully before use.

This is a highly sensitive kit for measuring rat albumin by ELISA.

[Advantage]

(1) This kit can measure rat albumin with a high sensitivity (50-1000ng/ml),

(2) With a small volume of sample (5 μl),

(3) With excellent reproducibility,

(4) And rapidly (2.5hours).

[Reagents]

A:	Anti-albumin-coated plate	96well(8x12)	x1
B:	Standard rat albumin solution (10μg/ml)	150μl	x1
C:	Buffer solution	60ml	x1
D:	HRP-conjugated antibody	100μl	x1
F:	Chromogenic substrate reagent (TMB)	12ml	x1
H:	Reaction stopper (1M H_2SO_4)	12ml	x1
I:	Concentrated washing buffer (10x)	100ml	x1

[Preparation of reagent solutions]

1 HRP-conjugated antibody
Dilute the original solution to 1:100 with buffer solution.

2. Chromogenic substrate solution
Use as it is without dilution.

3. Concentrated washing buffer
Dilute with distilled water to 1:10.

4. Standard albumin solution .
Prepare standard solutions as shown in a following example.

*An example of albumin standard solutions.

Albumin standard concentration (ng/ml)	1000	800	600	400	200	100	50	0
Albumin standard solution (μl)*	50	400	300	200	100	100	100	0
Buffer solution (μl)	450	100	100	100	100	100	100	100

*For 1000μg/ml use the attached original standard solution. From 800μl.ml on, use the one rank higher standard solution.

[Assay Procedure]

01) Rinse the anti-albumin coated plate by adding 250μl washing buffer(I) and discard 3 times, then strike the plate upside-down several times onto stacked sheets of paper towel, and remove the excess buffer.

02) Pipette 50μl of buffer solution into each well.

03) Shake the plate, and pipette 5μl sample or standard albumin solution.

04) Shake the plate, and incubate for 1 hour at room temperature.(20-25C).

05) Remove the reaction mixture, and rinse the plate 3 times as step 01.

06) Pipette 50μl of HRP-conjugated antibody solution into each well.

07) Shake the plate, and incubate for 1 hour at room temperature. (20-25C).

08) Remove the reaction mixture, and rinse the plate 3 times as step 01.

09) Pipette 50μl of chromoqenic substrate solution into each well.

10) Shake the plate, and incubate for 20 minutes at room temperature (20-25C).

11) Pipette 50μl of Reaction stopper(H) into each well.

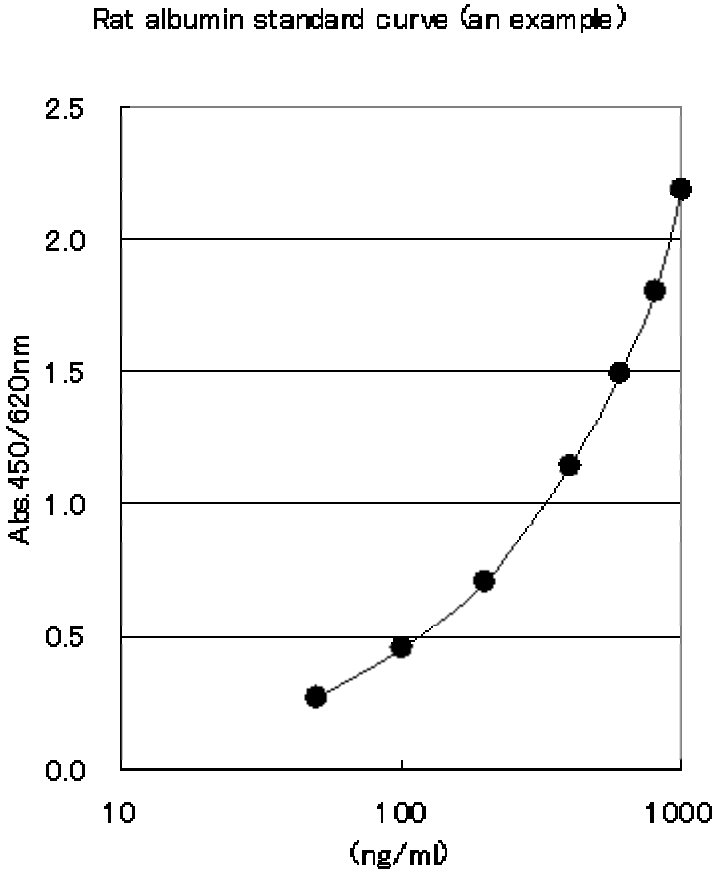
12) Shake the plate, and measure absorbance of each well at 450nm (sub-wavelength 620nm) by a plate reader within 30 minutes.

[Calculation of albumin concentration]

1. Prepare a standard curve albumin concentrations (ng/ml) on X-axis and absorbance on Y axis.

2. Read albumin concentrations of assay samples from their absorbance using the standard curve.

3. Calculate the albumin concentrations of original samples by multiplying the concentrations
with dilution factors.



Preparation of assay samples]

Regenerative medicine research field: Measurement of albumin in cultured cell/tissue and culture media.

Culture media --- Confirm the pH of the medium to be neutral, then confirm the parallelism of the dilution curve and the standard curve.

Tissue extracts --- Extracts should be properly diluted with the assay buffer. Confirm that the neutral pH and parallelism in a dilution test.

Kidney disease research field: Measurement of urinary albumin.

Urinary samples --- After removal of any precipitates by centrifugation. dilute the clear supernatant to more than 100 times with the assay buffer.. Then confirm the parallelism with the standard curve.

Measurement of albumin in blood samples.

Serum/plasma samples --- We recommend the use of heparin for plasma sampling. (e.g. Heparin 10-100μg/ml , final conc.)

Please, avoid using NaF-containing blood sampling tube, because fluoride ion is a peroxidase inhibitor, and may reduce the coloration even after washing.

Dilute serum/plasma samples to more than 10,000 times with the assay buffer. Then confirm the parallelism with the standard curve.

[Cross-reactivity]

Human albumin (10μg/ml), mouse albumin (10μg/ml), BSA (1mg/ml) and FCS (15%) are less than assay limit.

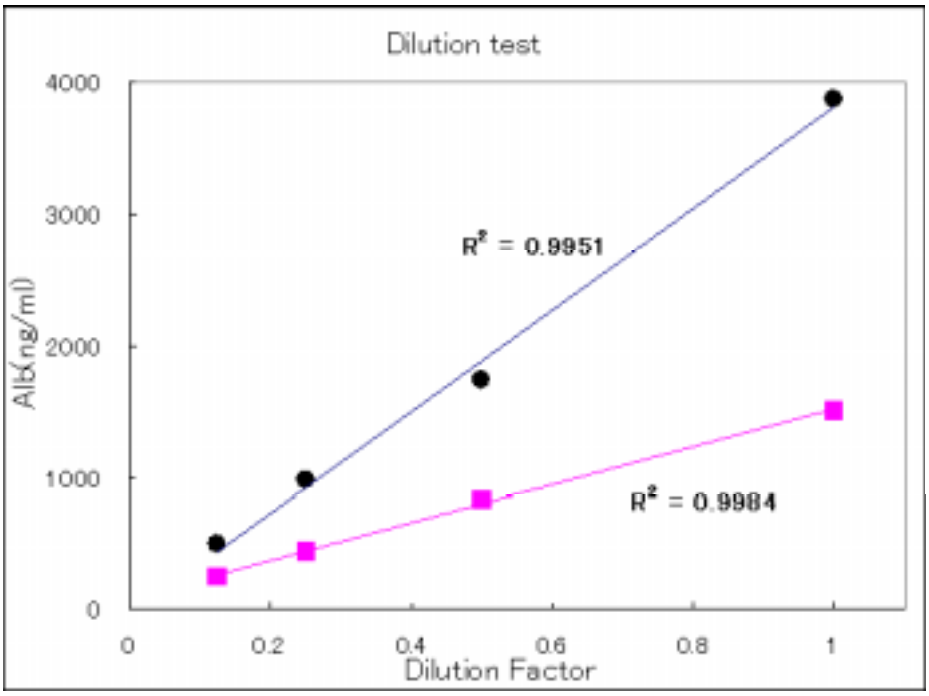
[Results of validity tests]

Reproducibility

Sample	Day 1	Day 2	Day 3	Mean	SD	CV(%)
3	32.1	33.4	33.8	33.1	0.895	2.71
4	482	474	490	482	7.70	1.60
5	2942	2915	3010	2956	49.1	1.66
				ng/ml		(n=3)

Precision

	Well No.							
Sample	1	2	3	4	5	Mean	SD	CV(%)
6	31.8	32.8	31.6	32.0	32.2	32.1	0.461	1.44
7	515	521	520	509	508	515	6.22	1.21
8	2911	2957	2812	2844	3052	2915	94.9	3.25
					ng/ml			



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