Mouse Total Angiotensinogen Assay Kit - IBL

96 Well

Please read carefully this instruction prior you use this assay kit.

INSTRUCTIONS FOR USE

This product is for research use only and is not intended for diagnostic use.

KIT COMPONENT

1	**Precoated plate:** (Anti- Mouse AGT (135) Rabbit IgG A.P.)	96Well x 1
2	**Labeled antibody conc.:** (30X) HRP conjugated Anti-Mouse AGT (405) Rabbit IgG Fab' A.P.)	0.4mL x 1
3	**Standard:** (Recombinant Mouse Angiotensinogen)	0.5mL x 2
4	**EIA buffer**	30mL x 1
5	**Solution for labeled antibody**	12mL x 1
6	**Chromogen:** TMB solution	15mL x 1
7	**Stop solution**	12mL x 1
8	**Wash buffer conc.**	50mL x 1

MEASURING SAMPLES

Mouse serum, EDTA-plasma, urine and cell culture supernatant.

PRINCIPLE

This kit is a solid phase sandwich ELISA (Enzyme-linked Immunosorbent Assay). As a primary antibody is coated on a plate, samples and standard are added into the wells for 1st reaction. After the reaction, HRP-conjugated secondary antibody is added into the wells for 2nd reaction. After washing away unbound the secondary antibody, Tetra Methyl Benzidine (TMB) is added to the wells and color develops.

OPERATING PRECATION

1 Test samples should be measured soon after collection. For storage of samples, store them frozen and do not repeat freeze/thaw cycles. Thaw the test samples at a low temperature and mix them completely before measurement.
2 Test samples should be diluted with "4, EIA buffer" contained in this kit.
3 Duplicate measurement of test samples and standards is recommended.
4 Standard curve should run for each assay.
5 Use test samples in neutral pH range. The contaminations of organic solvent may affect the measurement.
6 All reagents should be brought to room temperature (R.T.) and mixed completely and gently before use. After mixing them, make sure of no change in quality of the reagents.
7 Use only "8, Wash buffer conc." contained in this kit for washing the precoated plate. Insufficient washing may lead to the failure in measurement.
8 Fill the wash buffer each well, invert the plate and make sure the liquid is completely removed by shaking it off if you use a washing bottle. Repeat this washing process several times as instructed in order to avoid any insufficient washing process.
9 After remove the wash buffer, tapping the plate against a clean paper towel for completely removing the liquid from the wells and make sure the paper towel is not contact with inside of the wells in this process.
10 "6, Chromogen - TMB solution" should be stored in the dark due to its sensitivity against light. It should be also avoided contact with metals. Required quantity should be prepared into a collecting container for each use.
11 After adding TMB solution into the wells, the liquid in the wells gradually changes the color in blue. In this process the plate should be in dark. Remained TMB solution in the collecting container should not be returned into the original bottle of TMB solution to avoid contamination.
12 Measurement of O.D. should be done within 30 minutes after addition of "7, Stop solution".

OPERATION MANUAL AND DOSAGES

1. Materials needed but not supplied.

Plate reader	Micropipette and tip
Test tubes for dilution	Measuring cylinder and beaker
Deionized water	Plate washer
Paper towel	Collecting container
Incubator (37°C±1°C)	(i.e. clean disposable test tube)

2. Preparation

(1) Preparation of wash buffer

Dilute "8, Wash buffer conc." 40 fold with deionized water. The diluted one is used for the assay as a wash buffer. Adjust the required quantities if needed.

(2) Preparation of labeled antibody

Dilute "2, Labeled antibody conc." 30 fold with "5, Solution for labeled antibody" using a prepared collecting container.

(3) Preparation of standard

Add 0.5 mL of deionized water into the vial of "3, Standard" and completely dissolve it. Concentration of the standard is 20 ng/mL. The standards enclosed in this kit can be frozen and stored after reconstitution. However the freeze-thaw shall not be repeated.
Prepare 7 test tubes for dilution of the standard and adding 230 µL of the EIA buffer into each tube.

Put 230 µL of 20 ng/mL standard into the tube 10 ng/mL (Tube-1) and gently mix it. Afterword, put 230 µL of the mixed liquid of tube-1 into the tube 5 ng/mL (Tube-2) and gently mix it. Dilute two fold standard solution in series to set up 7 points of diluted standard between 10 ng/mL and 0.16 ng/mL.

Tube-1	10	ng/mL
Tube-2	5	ng/mL
Tube-3	2.5	ng/mL
Tube-4	1.25	ng/mL
Tube-5	0.63	ng/mL
Tube-6	0.31	ng/mL
Tube-7	0.16	ng/mL

(4) Preparation of test samples

Dilute test samples with "4, EIA buffer" contained in this kit as follows.
Rat serum or EDTA-plasma: more than 1,000 fold.
Rat urine: more than 16 fold.
Large volume of the EIA buffer (Mouse/Rat Angiotensinogen EIA buffer 100mL, Code No. 27413D100) is available with charge if required.

3 MEASUREMENT PROCEDURE

(1) Add test sample blank
Determine wells for test sample blank. Put 100µL each of "4, EIA buffer" into the wells.
(2) Add prepared test samples and standard
Put 100 µL prepared test samples and 100 µL prepared standard into appropriate wells.
(3) Incubation with plate lid (1st reaction).
(4) Washing
Wash the plate with the prepared wash buffer and remove all liquid.
(5) Add prepared labeled antibody
Put 100 µL prepared labeled antibody into the wells.
(6) Incubation with plate lid (2nd reaction).
(7) Washing
Wash the plate with the prepared wash buffer and remove all liquid completely.
(8) Add "6, Chromogen - TMB solution"
Put 100 µL the TMB solution into the wells.
(9) Incubation in dark
(10) Add "7, Stop solution"
Put 100 µL the Stop solution into the wells.
(11) Determination of optical density (O.D.)
Remove any dirt or drop of water on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, measure the both O.D. of standard and the test samples against a test sample blank.
Measurement wavelength: 450 nm. In case of 2 wavelengths:
Main wavelength is 450nm. Sub-wavelength is between 600 and 650 nm.

Table for measurement procedure

	Test samples	Standard	Test sample blank
Reagents	Test samples 100 µL	Diluted Standard 100 µL	EIA buffer 100 µL
1st reaction	Incubation for 60 minutes at 37°C with plate lid.		
Washing	4 times (wash buffer more than 350 µL)		
Labeled antibody	100 µL	100 µL	100 µL
2nd reaction	Incubation for 30 minutes at 37°C with plate lid.		
Washing	5 times (wash buffer more than 350 µL)		
TMB solution	100 µL	100 µL	100 µL
Chromogenic reaction	Incubation for 30 minutes at R.T. (shielded).		
Stop solution	100 µL	100 µL	100 µL
Measuring O.D.	450 nm / 600～650 nm		

CALCULATION OF TEST RESULT

1 Plot the concentration of the standard on the x-axis and its O.D. on the y-axis. Draw a standard curve by applying appropriate regression curve on each plot (i.e. quadratic regression of double logarithm conversion).

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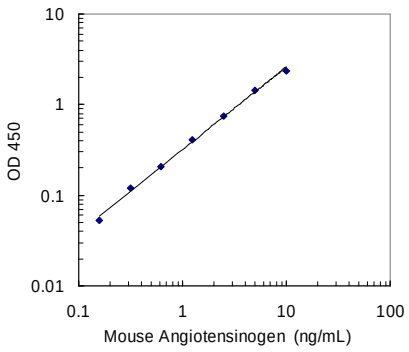
2 Read the concentration by applying the absorbance of the test samples on a standard curve.
3 Calculate the concentration of the test samples by multiplying dilution ratio of test samples on the value.

Example of standard curve and measured value

Standard (ng/mL)	O.D. (450nm)
10	2.371
5	1.451
2.5	0.753
1.25	0.415
0.63	0.207
0.31	0.119
0.16	0.053



PERFORMANCE AND CHARACTERISTICS

1 Sensitivity
0.03 ng/mL

2 Measurement range
0.16 ～ 10 ng/mL

3 Dilution linearity

Specimen	Titer (X)	Theoretical Value (ng/mL)	Measurement Value (ng/mL)	%
10 % FCS added RPMI-1640	4	2.50	2.35	94.0
	8	1.25	1.21	96.8
	16	0.63	0.62	98.4
Mouse Serum (BALB/c Mouse)	800	9.27	8.55	92.2
	1,600	4.83	4.68	96.9
	3,200	2.46	2.58	104.9
Mouse Plasma (EDTA) (BALB/c Mouse)	800	9.28	8.43	90.8
	1,600	4.70	4.72	100.4
	3,200	2.46	2.48	100.8
Mouse Urine (C57BL/6 Mouse)	16	0.84	0.59	70.2
	32	0.42	0.33	78.6
	64	0.22	0.17	77.3

4 Added recovery assay

Specimen	Theoretical Value (ng/mL)	Measurement Value (ng/mL)	%
10%FCS added RPMI-1640 (x4)	5.00	4.81	96.2
	2.50	2.63	105.2
	1.25	1.05	84.0
Mouse Serum (BALB/c Mouse) (x800)	9.70	8.72	89.9
	7.20	7.23	100.4
	5.95	5.71	96.0
Mouse Plasma (EDTA) (BALB/c Mouse) (x800)	9.45	8.91	94.3
	6.95	7.24	104.2
	5.70	5.95	104.4
Mouse Urine (C57BL/6 Mouse) (x16)	3.08	2.83	91.9
	1.83	1.59	86.9
	1.21	1.07	88.4

5 Intra-assay

Measurement value (ng/mL)	SD (ng/mL)	CV (%)	n
4.36	0.43	9.9	16
1.34	0.10	7.5	16
0.40	0.02	5.0	16

6 Inter-assay

Measurement value (ng/mL)	SD (ng/mL)	CV (%)	n
4.73	0.58	12.3	7
1.37	0.13	9.5	7
0.39	0.02	5.1	7

7 Specificity

Substance	Cross reactivity (%)
Mouse Angiotensinogen	100
Angiotensin I	≦0.1
Angiotensin II	≦0.1
Angiotensin III	≦0.1
Angiotensin IV	≦0.1
Angiotensin (1-7)	≦0.1
Angiotensin (1-9)	≦0.1
Mouse Albumin	≦0.1
Mouse IgG	≦0.1
Mouse Angiopoietin-like 3	≦0.1

PRECAUTION FOR INTENDED USE AND/OR HANDLING

1 Precaution for handling (Hazard prevention)

(1) Treat the components carefully and wash hands after handling it.
(2) “7, Stop solution” is a strong acid substance (1N Sulfuric acid). Therefore, it should be careful for the treatment and do not contact your skin and clothes with it. It also needs to pay attention to the disposal of it.

2 Precaution for intended use

(1) “3, Standard” is lyophilized products. It should be careful to open this vial.
(2) All reagents should be stored at 2 - 8°C.
(3) Precipitation can be seen in “4, EIA buffer”, “5, Solution for labeled antibody” and “8, Wash buffer conc.”, however, it does not affect its performance.
(4) Do not mix or replace the reagents with the reagents from a different lot or kit.
(5) Do not use expired reagents.

3 Precaution for disposal

(1) Dispose used materials after rinsing them with large quantity of water.

STORAGE AND THE TERM OF VALIDITY

Storage Condition: 2 - 8°C
The expiry date is specified on the outer box.

PACKAGE UNIT AND PRODUCT NUMBER

Package unit: 96 Well
Product number: 27413

REFERENCES

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6. Kobori H, Nangaku M, Navar LG, Nishiyama A. The intrarenal renin-angiotensin system: from physiology to the pathobiology of hypertension and kidney disease.Pharmacol Rev. 2007 Sep;59(3):251-87.
7. Kobori H, Katsurada A, Miyata K, Ohashi N, Satou R, Saito T, Hagiwara Y, Miyashita K, Navar LG. Determination of plasma and urinary angiotensinogen levels in rodents by newly developed ELISA. Am J Physiol Renal Physiol. 2008 May;294(5):F1257-63.

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