Code No. 27412

Human Total Angiotensinogen Assay Kit - IBL

INTRODUCTION

Angiotensinogen is the precursor of angiotensin and is cleaved into angiotensin I and II in the renin-angiotensin system, and it has long been reported to play an important role in controlling blood pressure. In recent years interest related to the role of the renin-angiotensin system in arterial pressure control and the pathophysiology of hypertension has been shifting to its local role in various tissues. Among the studies urinary excretion of angiotensinogen in a rat model of angiotensin II (AII)-dependent hypertension has been reported to be a marker of the activity of the local intrarenal renin-angiotensin system. Intrarenal AII increases to an extent in AII-dependent hypertension that cannot be explained by the plasma AII equilibration alone, and two mechanisms, an increase in intracellular uptake of AII and an increase in intrarenal expression of angiotensinogen, have been proposed to explain it.
This product is a complete kit for the quantitative determination of human angiotensinogen in serum, EDTA-plasma, urine or cell culture media.

PRINCIPLE

This kit is a solid phase sandwich ELISA using 2 kinds of high specific antibodies. Tetra Methyl Benzidine (TMB) is used as a coloring agent (Chromogen). The strength of coloring is proportional to the quantities of human angiotensinogen.

MEASUREMENT RANGE

0.31 ～ 20 ng/mL
(6.0 – 384.6 pmol/L, as molecular weight of human angiotensinogen is 52 kDa)

INTENDED USE

For research use only, not for use in diagnostic procedures.

- This kit can measure human angiotensinogen in serum, EDTA-plasma, urine or cell culture media. And dilute test samples with special “4, EIA buffer” as necessary before assay.
- The recommended dilution rate for urine samples is 4 - 8 fold.
- The recommended dilution rate for serum or EDTA-plasma samples is about 10,000-fold.
- The recommended dilution for cell culture media samples is various by using cells, therefore, the dilution rate should be optimized by each laboratories.

KIT COMPONENT

1	Precoated plate : Anti-Human AGT (72) Rabbit IgG Affinity Purify	96Well x 1
2	Labeled antibody Conc. : (30X) HRP conjugated Anti- Human AGT (601) Mouse IgG Fab’ Affinity Purify	0.4mL x 1
3	Standard : Angiotensinogen from human plasma	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

OPERATION MANUAL

1. Materials needed but not supplied

- Plate reader (450nm)
- Micropipette and tip
- Graduated cylinder and beaker
- Deionized water
- Incubator (37°C ± 1°C)
- Graph paper (log/log)
- Paper towel
- Tube for dilution of Standard
- Washing bottle for precoated plate
- Disposable test tube for “2, Labeled antibody Conc.” and “6, Chromogen”

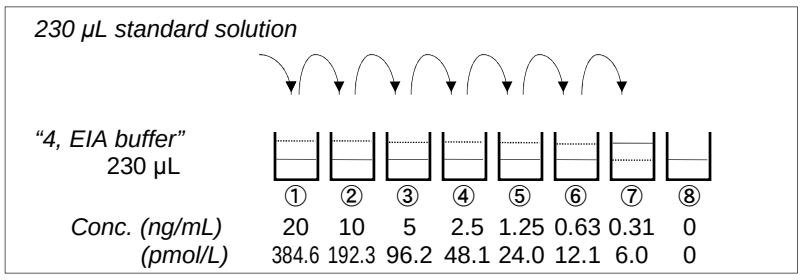
2. Preparation

1) Preparation of wash buffer
“8, Wash buffer Conc.” is a concentrated (40X) buffer. Adjust the temperature of “8, Washing buffer Conc.” to room temperature and then, mix it gently and completely before use. Dilute 50 mL of “8, Wash buffer Conc.” with 1,950 mL of deionized water and mix it. This is the wash buffer for use. This prepared wash buffer shall be stored in refrigerator and used within 2 weeks after dilution.
2) Preparation of Labeled antibody
“2, Labeled antibody Conc.” is a concentrated (30X). Dilute “2, Labeled antibody Conc.” with “5, Solution for Labeled antibody” in 30 times according to required quantity into a disposable test tube. Use this resulting solution as Labeled antibody.
Example)
In case you use one strip (8 well), the required quantity of Labeled antibody is 800 µL. (Dilute 30 µL of “2, Labeled antibody Conc.” with 870 µL of “5, Solution for Labeled antibody” and mix it. And use the resulting solution by 100 µL in each well.)
This operation should be done just before the application of Labeled antibody.
The remaining “2, Labeled antibody Conc.” should be stored at 4℃ in firmly sealed vial.
3) Preparation of Standard
Put just 0.5 mL of deionized water into the vial of “3, Standard” and mix it gently and completely. This solution is 40 ng/mL (769.2 pmol/L) human angiotensinogen standard.
4) Dilution of Standard
Prepare 8 tubes for dilution of “3, Standard”. Put 230 µL each of “4, EIA buffer” into the tube.
Specify the following concentration of each tube.”

Tube-1	20 ng/mL	(384.6 pmol/L)
Tube-2	10 ng/mL	(192.3 pmol/L)
Tube-3	5 ng/mL	(96.2 pmol/L)
Tube-4	2.5 ng/mL	(48.1 pmol/L)
Tube-5	1.25 ng/mL	(24.0 pmol/L)
Tube-6	0.63 ng/mL	(12.1 pmol/L)
Tube-7	0.31 ng/mL	(6.0 pmol/L)
Tube-8	0 ng/mL	(0 pmol/L) (Test Sample Blank)

Put 230 µL of Standard solution into tube-1 and mix it gently. Then, put 230 µL of tube-1 mixture into tube-2. Dilute two times standard solution in series to set up 7 points of diluted standard between 20 ng/mL (384.6 pmol/L) and 0.31 ng/mL (6.0 pmol/L). Tube-8 is the test sample blank as 0 ng/mL (0 pmol/L).

See following picture.



5) Dilution of test sample
Test sample should be diluted with special “4, EIA buffer” suitably.
If the concentration of Human Angiotensinogen in samples may not be estimated in advance, the pre-assay with several different dilutions will be recommended to determine the proper dilution of samples.
When “4, EIA buffer” in kit is not enough for dilution, customers can purchase additional kit component (30 mL, Code No. 27412D) or large volume EIA buffer for Human Angiotensinogen (100 mL, Code No. 27412D100).

6) Example of sample dilution
x10,000 dilution of serum or EDTA-plasma
At first, add 990 µL of “4, EIA buffer” to 10 µL of sample and mix it gently and completely. Then, this solution is “100-fold diluted sample”.
Next, add 990 µL of “4, EIA buffer” to 10 µL of the “100-fold diluted sample” and mix it again. Then, this resulting solution is 10,000-fold diluted sample and use it for determination.

3. Measurement procedure

All reagents shall be brought to room temperature approximately 30 minutes before use. Then mix it gently and completely before use. Make sure of no change in quality of the reagents. Standard curve shall be prepared simultaneously with the measurement of test samples.

Reagents	Test Sample	Standard	Test Sample Blank	Reagent Blank
	Test sample 100 µL	Diluted standard (Tube 1-7) 100 µL	EIA buffer (Tube-8) 100 µL	EIA buffer 100 µL
Incubation for 60 minutes at 37°C with plate lid				
4 times (wash buffer more than 350 µL)*				
Labeled Antibody	100 µL	100 µL	100 µL	-
Incubation for 30 minutes at 37°C with plate lid				
5 times (wash buffer more than 350 µL)*				
Chromogen	100 µL	100 µL	100 µL	100 µL
Incubation for 30 minutes at room temperature (shielded)				
Stop solution	100 µL	100 µL	100 µL	100 µL
Read the plate at 450nm against a Reagent Blank within 30 minutes after addition of Stop solution.				

1) Determine wells for reagent blank. Put 100 µL each of “4, EIA buffer” into the wells.
2) Determine wells for test sample blank, test sample and diluted standard. Then, put 100 µL each of test sample blank (tube-8), test sample and dilutions of standard (tube-1-7) into the appropriate wells.
3) Incubate the precoated plate for 60 minutes at 37°C after covering it with plate lid.
4) Wash the plate with the prepared wash buffer and remove all liquid.*
5) Pipette 100 µL of labeled antibody solution into the wells of test samples, diluted standard and test sample blank.
6) Incubate the precoated plate for 30 minutes at 37°C after covering it with plate lid.
7) Wash the plate with the prepared wash buffer and remove all liquid.*
8) Take the required quantity of “6, Chromogen” into a disposable test tube. Then, pipette 100 µL from the test tube into the wells. Please do not return the rest of the test tube to “6, Chromogen” bottle to avoid contamination.
9) Incubate the precoated plate for 30 minutes at room temperature in the dark. The liquid will turn blue by addition of “6, Chromogen”.
10) Pipette 100 µL of “7, Stop solution” into the wells. Mix the liquid by tapping the side of precoated plate. The liquid will turn yellow by addition of “7, Stop solution”.
11) Remove any dirt or drop of water on the bottom of the precoated plate and confirm there is no bubble on the surface of the liquid. Then, run the plate reader and conduct measurement at 450 nm against a reagent blank.
The measurement shall be done within 30 minutes after addition of “7, Stop solution”.

SPECIAL ATTENTION

1) Test samples should be measured soon after collection. For the storage of test 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 have to be diluted with “4, EIA buffer” suitably.
3) Duplicate measurement of test samples and standard is recommended.
4) Use test samples in neutral pH range. The contaminations of organic solvent may affect the measurement.
5) Use only wash buffer contained in this kit for washing the precoated plate. Insufficient washing may lead to the failure in measurement.

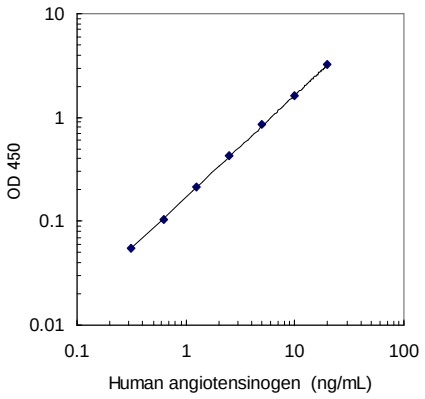
6) Remove the wash buffer completely by tapping the precoated plate on paper towel. Do not wipe wells with paper towel.
7) "6, Chromogen" should be stored in the dark due to its sensitivity against light. "6, Chromogen" should be avoided contact with metals.
8) Measurement should be done within 30 minutes after addition of "7, Stop solution".

CALCULATION OF TEST RESULT

Subtract the absorbance of test sample blank from all data, including standards and unknown samples before plotting. Plot the subtracted absorbance of the standards against the standard concentration on log-log graph paper. Draw the best smooth curve through these points to construct the standard curve. Read the concentration for unknown samples from the standard curve.

Example of standard curve

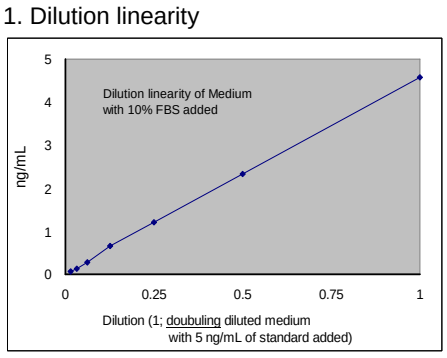
Conc. ng/mL (pmol/L)	Absorbance (450nm)
20 (384.6)	3.221
10 (192.3)	1.604
5 (96.2)	0.856
2.5 (48.1)	0.426
1.25 (24.0)	0.216
0.63 (12.1)	0.108
0.31 (6.0)	0.058
0 (Test Sample Blank)	0.003

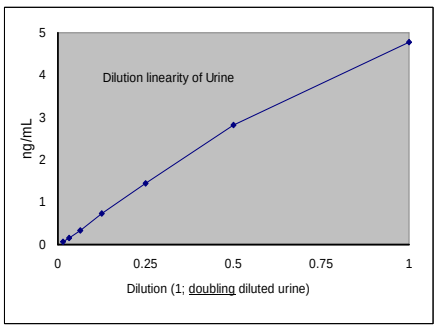


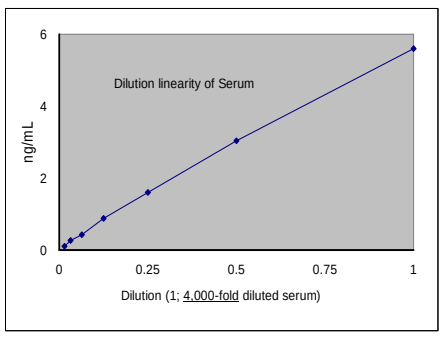
* The typical standard curve is shown above. This curve can not be used to derive test results. Please run a standard curve for each assay.

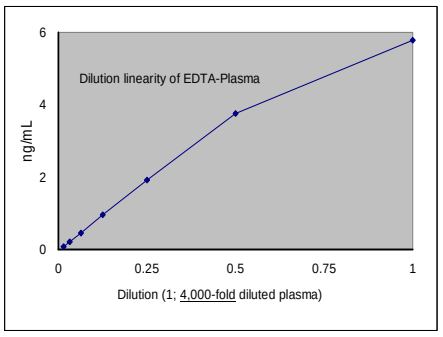
PERFORMANCE CHARACTERISTICS

1. Dilution linearity









2. Added Recovery Assay

Specimen	Additive Amount (ng/mL)	Theoretical Value (ng/mL)	Measured Value (ng/mL)	%
10% FBS added Medium (x2)	5.00	5.00	4.24	84.8
	2.50	2.50	2.17	86.8
	1.25	1.25	1.07	85.6
Human Serum (x8,000)	5.00	9.39	8.03	85.5
	2.50	6.89	6.03	87.5
	1.25	5.64	5.07	89.9
Human Plasma (EDTA) (x8,000)	5.00	9.29	7.70	82.9
	2.50	6.79	6.50	95.7
	1.25	5.54	4.98	89.9
Human Urine (x8)	5.00	7.47	6.54	87.6
	2.50	4.97	4.69	94.4
	1.25	3.72	3.38	90.9

3. Intra - Assay

Mean Value (ng/mL)	SD (ng/mL)	CV (%)	n
8.81	0.39	4.4	24
2.14	0.11	5.1	24
0.73	0.04	5.5	24

4. Inter - Assay

Mean Value (ng/mL)	SD (ng/mL)	CV (%)	n
8.69	0.13	1.5	3
2.14	0.02	0.9	3
0.69	0.04	5.8	3

5. Specificity

Substance	Cross-reactivity
Human 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 %
Human albumin	< 0.1 %
Human IgG	< 0.1 %
Human Angiopoietin-like 3	< 0.1 %

6. Sensitivity

0.03 ng/mL
The sensitivity for this kit was determined using the guidelines under the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols. (National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.)

PRECAUTION FOR INTENDED USE AND/OR HANDLING

1. All reagents should be stored at 2 - 8°C. All reagents shall be brought to room temperature approximately 30 minutes before use.
2. "3, Standard" is lyophilized products. Be careful to open this vial.
3. "7, Stop solution" is a strong acid substance. Therefore, be careful not to have your skin and clothes contact "7, Stop solution" and pay attention to the disposal of "7, Stop solution".
4. Dispose used materials after rinsing them with large quantity of water.
5. Precipitation may occur in "2, Labeled antibody Conc.", "4, EIA buffer" or "8, Wash buffer Conc.", however, there is no problem in the performance.
6. Wash hands after handling reagents.
7. Do not mix the reagents with the reagents from a different lot or kit.
8. Do not use expired reagents.
9. This kit is for research purpose only. Do not use for clinical diagnosis.

STORAGE AND THE TERM OF VALIDITY

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

REFERENCE

1. Kobori H, Harrison-Bernard LM, Navar LG. Expression of angiotensinogen mRNA and protein in angiotensin II-dependent hypertension. J Am Soc Nephrol. 2001 Mar;12(3):431-9.
2. Kobori H, Harrison-Bernard LM, Navar LG. Enhancement of angiotensinogen expression in angiotensin II-dependent hypertension. Hypertension. 2001 May;37(5):1329-35.
3. Kobori H, Harrison-Bernard LM, Navar LG. Urinary excretion of angiotensinogen reflects intrarenal angiotensinogen production. Kidney Int. 2002 Feb;61(2):579-85.
4. Kobori H, Nishiyama A, Harrison-Bernard LM, Navar LG. Urinary angiotensinogen as an indicator of intrarenal Angiotensin status in hypertension. Hypertension. 2003 Jan;41(1):42-9.
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6. Katsurada A, Hagiwara Y, Miyashita K, Satou R, Miyata K, Ohashi N, Navar LG, Kobori H. Novel sandwich ELISA for human angiotensinogen.Am J Physiol Renal Physiol. 2007 Sep;293(3):F956-60.
7. 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.

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