Human Intact 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-Human AGT(A1) Rabbit IgG A.P)	96Well x 1
2	**Labeled antibody conc.:** ((30X) HRP conjugated Anti- Human AGT(601) Mouse IgG Fab')	0.4mL x 1
3	**Standard:** (Human 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

Human serum, EDTA plasma and urine(Recommend to use spot urine samples).

※Caution for measuring samples.

1) It is recommended to add enzyme inhibitor for collecting serum, EDTA plasma and urine (spot urine).
Adding 1/100 volume of Protease Inhibitor Cocktail (SIGMA P8340).
2) Serum and EDTA plasma should be separated immediately after blood collection. It is essential to add the enzyme inhibitor above if serum and EDTA plasma are not separated immediately after blood collection.
3)The samples should be measured within 3 hours after the separation. The separated serum and EDTA-plasma should be frozen and stored if the samples are not be measured within 3 hours after the separation.

It cannot be guaranteed for the accuracy for obtained data if the samples are not measured followed by above procedure.

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 Using a plate washer is recommended (wait time zero second). It should be washed by a plate washer immediately after each reaction. If you use a washing bottle instead of a plate washer, after filling wash buffer in each well, immediately turn the plate upside down and shake it off to completely remove the wash buffer. Repeat the number of times of wash defined in a table for measurement procedure described in section 3. It should be properly washed off as instructed in order to avoid any insufficient wash.
9 Carefully tap the plate against a clean paper towel without contacting with inside of each well to completely remove the washing buffer after repeated the determined number of wash.
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 or washing bottle
Paper towel	Collecting container
Incubator(37℃±1℃)	(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.

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 100µL the mixed solution in each well.)
This operation should be done just before applying labeled antibody.
The remaining “2, Labeled antibody Conc.” should be stored at 4°C in a firmly sealed vial.

(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 36 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 36 ng/mL standard into the tube 18 ng/mL (Tube-1) and gently mix it. Afterword, put 230 µL of the mixed liquid of tube-1 into the tube 9 ng/mL (Tube-2) and gently mix it. Dilute two fold standard solution in series to set up 7 points of diluted standard between 18 ng/mL and 0.28 ng/mL.

Tube-1	18	ng/mL
Tube-2	9	ng/mL
Tube-3	4.5	ng/mL
Tube-4	2.25	ng/mL
Tube-5	1.13	ng/mL
Tube-6	0.56	ng/mL
Tube-7	0.28	ng/mL

(4) Preparation of test samples
Dilute test samples with “4, EIA buffer” contained in this kit as follows.

Human serum	: 4000 fold
Human EDTA plasma	: 4000 fold
Human urine	: 4 fold

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 EIA buffer for Human Intact Angiotensinogen (100 mL,Code No. 27742D100) .

(5) Example of sample dilution
X4,000 dilution of serum or EDTA-plasma
Prepare 2 tubes for dilution of test sample and put 156 µL of “4, EIA buffer” into the 1st tube. Then, add 4 µL of sample into the tube and mix it gently and completely for making 40-fold diluted sample.
Next, put 396 µL of “4, EIA buffer into the 2nd tube, then add 4 µL of the 40-fold diluted sample into the tube and mix it well for making 0.4mL of 4,000-fold diluted sample for determination.

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 (Refer to No. 8 and 9 described in OPERATING PRECATION.)
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 (Refer to No. 8 and 9 described in OPERATING PRECATION.)
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:

Manufacturer: Immuno-Biological Laboratories Co., Ltd.

Main wavelength is 450nm. Sub-wavelength is between 600 and 650 nm.

Table for measurement procedure

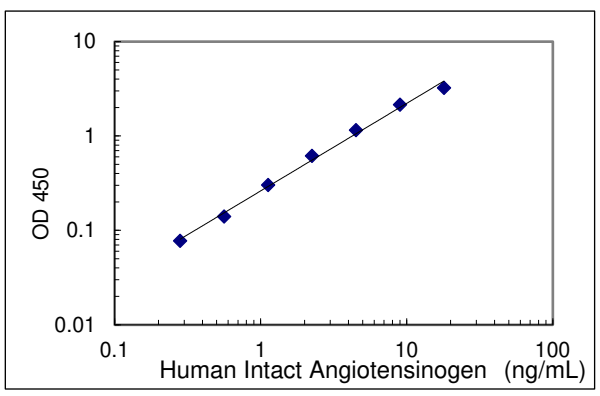
	Test samples	Standard	Test sample blank
Reagents	Test samples 100 µL	Diluted Standard 100 µL	EIA buffer 100 µL
1st reaction	Incubation for 60 minutes at 37℃ with plate lid		
Washing	4 times (wash buffer more than 350 µL) (Refer to No. 8 and 9 described in OPERATING PRECATION.)		
Labeled antibody	100 µL	100 µL	100 µL
2nd reaction	Incubation for 30 minutes at 37℃ with plate lid		
Washing	5 times (wash buffer more than 350 µL) (Refer to No. 8 and 9 described in OPERATING PRECATION.)		
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).
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)
18.	3.237
9.	2.149
4.5	1.151
2.25	0.615
1.13	0.302
0.56	0.140
0.28	0.077

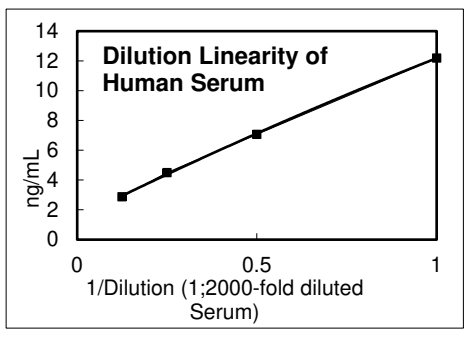


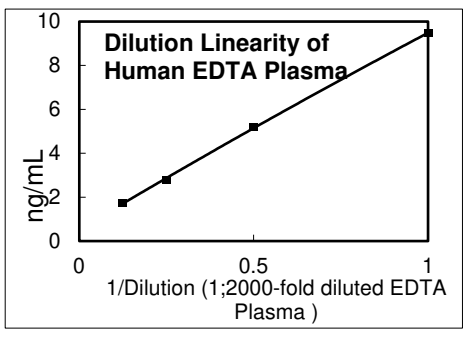
PERFORMANCE AND CHARACTERISTICS

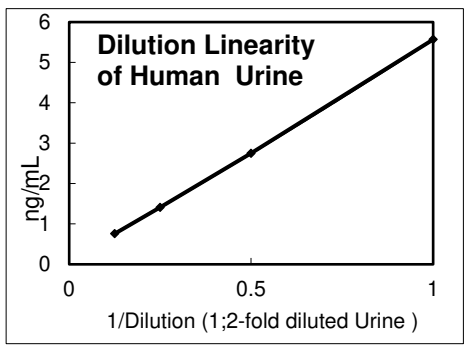
1 Sensitivity
0.093 ng/mL (Calculated by NCCLS method using the standard.)

2 Measurement range
0.28 ～ 18 ng/mL

3 Dilution linearity







4 Added recovery assay

Specimen	Additive Amount (ng/mL)	Theoretical Value (ng/mL)	Measurement Value (ng/mL)	%
Human Serum (x10)	0.56	5.37	4.85	90.3%
	0.28	5.09	4.77	93.7%
	0.14	4.95	4.74	95.8%
Human EDTA-Plasma (x10)	0.56	3.87	3.77	97.4%
	0.28	3.59	3.60	100.3%
	0.14	3.45	3.37	97.7%
Human Urine (x10)	2.25	5.99	6.19	103.3%
	1.13	4.86	4.96	102.1%
	0.56	4.30	4.30	100.0%

5 Intra-assay

Measurement value (ng/mL)	SD(ng/mL)	CV (%)	n
9.65	0.71	7.4	24
4.49	0.49	10.9	24
2.33	0.21	9.0	24

6 Inter-assay

Measurement value (ng/mL)	SD (ng/mL)	CV (%)	n
8.54	0.70	8.2	7
4.23	0.42	9.9	7
2.06	0.17	8.3	7

7 Specificity
Specifically detect Human Intact Angiotensinogen.
Angiotensinogen taken apart doesn't detect it in renin.

8 Interfering Substances
Hemolyzed hemoglobin does not affect on the value of measurement up to 4900 mg/dL.
Free bilirubin does not affect on the value of measurement up to 6.0 mg/dL.
Conjugated bilirubin does not affect on the valueof measurement up to 207 mg/dL.
Chyle does not affect on the value of measurement up to 2062.5 FTU.

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: 27742

CONTACT DETAILS

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