

# Human Total Angiotensinogen Assay Kit – IBL

96 Well

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

## INSTRUCTIONS FOR USE

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

### KIT COMPONENT

1	<b>Precoated plate:</b> (Anti- Human AGT (72) Rabbit IgG A.P.)	96Well x 1
2	<b>Labeled antibody conc.:</b> (30X) HRP conjugated Anti-Human AGT (601) Mouse IgG MoAb Fab' A.P.)	0.4mL x 1
3	<b>Standard:</b> (Human Angiotensinogen*)	0.5mL x 2
4	<b>EIA buffer</b>	30mL x 1
5	<b>Solution for labeled antibody</b>	12mL x 1
6	<b>Chromogen:</b> TMB solution	15mL x 1
7	<b>Stop solution</b>	12mL x 1
8	<b>Wash buffer conc.</b>	50mL x 1

### MEASURING SAMPLES

Human serum, EDTA-plasma, urine (Recommend to use spot urine samples) and cell culture supernatant.

#### ※Precaution of Urine Sample Handling\*

Angiotensinogen in urine samples is extremely unstable. As it is sensitive to temperature changes and may be rapidly deteriorated.

Please immediately measure the samples after urine collection or store the samples at -80°C after the collection.

Please also note that the measurement value might be decreased in the result leaving urine samples at room temperature (pooled samples).

### 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 1<sup>st</sup> reaction. After the reaction, HRP-conjugated secondary antibody is added into the wells for 2<sup>nd</sup> reaction. After washing away unbound the secondary antibody, Tetra Methyl Benzidine (TMB) is added to the wells and color develops.

### OPERATING PRECATION

- 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.
- Test samples should be diluted with "4, EIA buffer" contained in this kit.
- Duplicate measurement of test samples and standards is recommended.
- Standard curve should run for each assay.
- Use test samples in neutral pH range. The contaminations of organic solvent may affect the measurement.
- 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.
- Use only "8, Wash buffer conc." contained in this kit for washing the precoated plate. Insufficient washing may lead to the failure in measurement.
- Wash the plate immediately after each reaction using by a plate washer with setting wait time zero second. The O.D. value tends to be lower if washing time is getting longer. If you use a multichannel pipette or a washing bottle due to no availability of any plate washer, filling wash buffer in each well and 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.
- 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.
- "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.
- 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.
- 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

- 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.

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

- 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 40 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 40 ng/mL standard into the tube 20 ng/mL (Tube-1) and gently mix it. Afterword, put 230 µL of the mixed liquid of tube-1 into the tube 10 ng/mL (Tube-2) and gently mix it. Dilute two fold standard solution in series to set up 7 points of diluted standard between 20 ng/mL and 0.31 ng/mL.

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)

- Preparation of test samples  
Dilute test samples with "4, EIA buffer" contained in this kit as follows.  
Human serum or EDTA-plasma: about 10,000 fold.  
Human urine: 4 ~ 8 fold  
Cell culture supernatant: the dilution rate should be optimized by each laboratories. Large volume of the EIA buffer (Human Angiotensinogen EIA buffer 100mL, Code No. 27412D100) is available with charge if required.

Example of sample : x10,000 dilution of serum or EDTA-plasma  
Prepare 2 tubes for dilution of test sample. Put 990 µL each of "4, EIA buffer" into the tube.  
At first, add 10 µL of test sample into the tube and mix it gently and completely. Then, this solution is "100-fold diluted sample".  
Next, add 10 µL of the "100-fold diluted sample" into the next tube and mix it again. Then, this resulting solution is 10,000-fold diluted sample and use it for determination.

### 3 MEASUREMENT PROCEDURE

- Add test sample blank  
Determine wells for test sample blank. Put 100µL each of "4, EIA buffer" into the wells.
- Add prepared test samples and standard  
Put 100 µL prepared test samples and 100 µL prepared standard into appropriate wells.
- Incubation with plate lid (1st reaction).
- Washing  
Wash the plate with the prepared wash buffer and remove all liquid.
- Add prepared labeled antibody  
Put 100 µL prepared labeled antibody into the wells.
- Incubation with plate lid (2<sup>nd</sup> reaction).
- Washing  
Wash the plate with the prepared wash buffer and remove all liquid completely.
- Add "6, Chromogen - TMB solution"  
Put 100 µL the TMB solution into the wells.
- Incubation in dark
- Add "7, Stop solution"  
Put 100 µL the Stop solution into the wells.
- 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

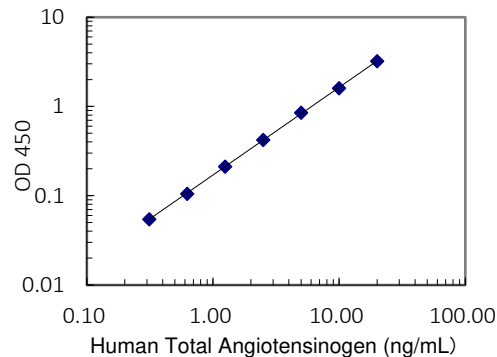
	Test samples	Standard	Test sample blank
Reagents	Test samples 100 µL	Diluted Standard 100 µL	EIA buffer 100 µL
1 <sup>st</sup> reaction	Incubation for 60 minutes at 37°C with plate lid.		
Washing	4 times (wash buffer more than 350 µL) (Refer to No. 8 and 9 described in OPERATING PRECATION.)		
Labeled antibody	100 µL	100 µL	100 µL
2 <sup>nd</sup> reaction	Incubation for 30 minutes at 37°C 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**

- 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).
- Read the concentration by applying the absorbance of the test samples on a standard curve.
- 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 (pmol/L)	O.D. (450nm)
20. (384.6)	3.218
10. (192.3)	1.601
5. (96.2)	0.853
2.5 (48.1)	0.423
1.25 (24.0)	0.213
0.63 (12.1)	0.105
0.31 (6.0)	0.055

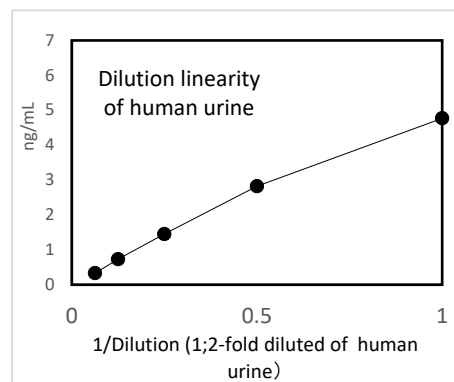
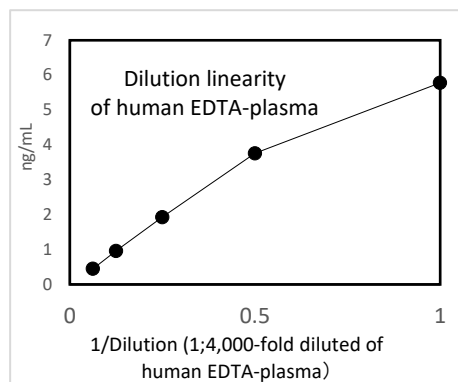
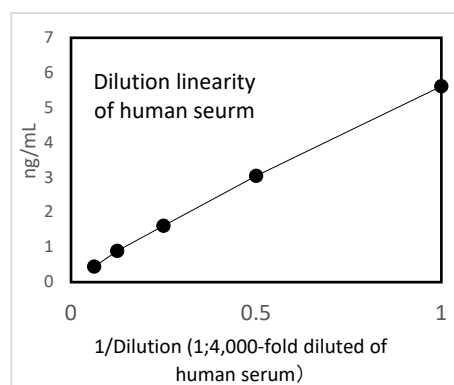
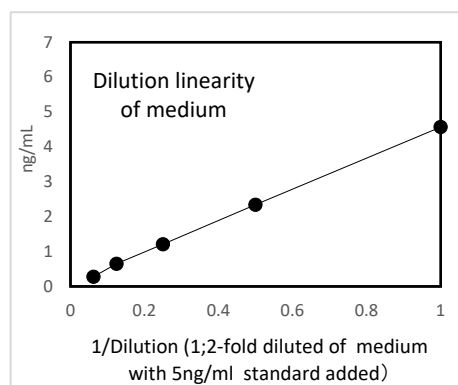

**PERFORMANCE AND CHARACTERISTICS**
**1 Sensitivity**

0.03 ng/mL (Calculated by NCCLS method using the standard.)

**2 Measurement range**

0.31 ~ 20 ng/mL

(6.0 ~384.6 pmol/L, as molecular weight of human angiotensinogen is 52 kDa)

**3 Dilution linearity**

**4 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 EDTA-plasma (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

**5 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

**6 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

**7 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 Angiotensin-like 3	< 0.1 %

**PRECAUTION FOR INTENDED USE AND/OR HANDLING**
**1 Precaution for handling (Hazard prevention)**

- Treat the components carefully and wash hands after handling it.
- "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**

- "3, Standard" is lyophilized products. It should be careful to open this vial.
- All reagents should be stored at 2 - 8°C.
- 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.
- Do not mix or replace the reagents with the reagents from a different lot or kit.
- Do not use expired reagents.

**3 Precaution for disposal**

- 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.

**REFERENCE**

- 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.
- Kobori H, Harrison-Bernard LM, Navar LG. Enhancement of angiotensinogen expression in angiotensin II-dependent hypertension. *Hypertension.* 2001 May;37(5):1329-35.
- Kobori H, Harrison-Bernard LM, Navar LG. Urinary excretion of angiotensinogen reflects intrarenal angiotensinogen production. *Kidney Int.* 2002 Feb;61(2):579-85.
- 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.
- Kobori H, Prieto-Carrasquero MC, Ozawa Y, Navar LG. AT1 receptor mediated augmentation of intrarenal angiotensinogen in angiotensin II-dependent hypertension. *Hypertension.* 2004 May;43(5):1126-32.
- 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.
- 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.

**PACKAGE UNIT AND PRODUCT NUMBER**

 Package unit: 96 Well  
 Product number: 27412

**CONTACT DETAILS**

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