ab325899 – Human AGT SimpleStep ELISA® Kit, Chemiluminescent

For the quantitative measurement of AGT in human serum, plasma (heparin), plasma (EDTA), plasma (citrate), cell culture supernatant, and tissue extract.
For research use only - not intended for diagnostic use.
Patent pending.

For overview, typical data and additional information please visit: www.abcam.com/ab325899

Storage and Stability: Store kit at 2-8°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Standard Preparation and Reagent preparation sections.

Limitations: All data, except typical standard curve and sensitivity were collected using the colorimetric version of this kit (ab287170).

Materials Supplied

Item	Quantity 1 x 96 tests	Storage Condition
Human AGT Capture Antibody 10X	600 µL	+4°C
Human AGT Detector Antibody 10X	600 µL	+4°C
Human AGT Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent 4BI	6 mL	+4°C
Cell Extraction Buffer PTR 5X	10 mL	+4°C
Sample Diluent NS	50 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
ChemiHRP Reagent A	3 mL	+4°C
ChemiHRP Reagent B	3 mL	+4°C
SimpleStep Pre-Coated Black 96-Well Microplate	96 Wells	+4°C
Plate Seal	1	+4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

Luminometer with the following settings: 0.5-1 second/well read time; summation mode (all wavelengths).
Method for determining protein concentration (BCA assay recommended).
Deionized water.
Multi- and single-channel pipettes.
Tubes for standard dilution.
Orbital microplate shaker for all incubation steps: capable of 750 rpm shaking speed.
Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.

Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

1X Wash Buffer PT: Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

1X Cell Extraction Buffer PTR (For cell and tissue extracts only): Prepare 1X Cell Extraction Buffer PTR by diluting Cell Extraction Buffer PTR 5X to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 8 mL deionized water and 2 mL Cell Extraction Buffer PTR 5X. Mix thoroughly and gently. If required protease inhibitors can be added.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 4BI. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent 4BI. Mix thoroughly and gently.

Lumi HRP Development Solution: Just prior to use, prepare Lumi HRP Development Solution by mixing equal volume of the ChemiHRP Reagent A and the ChemiHRP Reagent B. To make 3 mL of the Lumi HRP Development Solution combine 1.5 mL of ChemiHRP Reagent A and 1.5 mL of ChemiHRP Reagent B. Mix thoroughly and gently by inversion or slow pipetting (Avoid shaking or vortexing). Protect the prepared solution from light until use.

Standard Preparation

Always prepare a fresh set of standards for every use. Discard working standard dilutions after use as they do not store well. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

1. Reconstitute the **AGT** protein standard by adding the volume indicated on the protein vial label. For **serum, plasma and cell culture supernatant samples measurements**, use Sample Diluent NS. For **tissue extract samples measurements**, use 1X Cell Extraction Buffer PTR. Hold at room temperature for 10 minutes and mix thoroughly and gently. This is the 12,000 pg/mL **Stock Standard #1** Solution.
2. Label seven tubes, Standards 1 – 7.
3. Use the same Sample Diluent as used to resuspend the Stock Standard to prepare the standard curve. Add 150 µL of Sample Diluent into numbers 2-7.
4. Use the **Stock Standard #1** to prepare the following dilution series. Standard #7 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	**Stock Standard #1**	150	0	12,000	12,000
2	**Stock Standard #1**	75	150	12,000	4,000
3	Standard#2	75	150	4,000	1,333.3
4	Standard#3	75	150	1,333.3	444.4
5	Standard#4	75	150	444.4	148.2
6	Standard#5	75	150	148.2	49.4
7	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum	1:320,000 – 1:20,000
Plasma – Citrate	1:320,000 – 1:20,000
Plasma – EDTA	1:160,000 – 1:20,000
Plasma – Heparin	1:320,000 – 1:20,000
Cell Culture Media*	≤ 100%
Lung Tissue Extract	1.56 µg/mL – 25 µg/mL

*Media is RPMI without FBS.

Serum Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute samples at least 1:20,000 into Sample Diluent NS and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles

Plasma Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples at least 1:20,000 into Sample Diluent NS and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Cell Culture Supernatants Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants and assay. Or dilute samples into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Note: This kit is not compatible with media containing bovine serum.

Preparation of extracts from tissue homogenates Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended). Homogenize 100 to 200 mg of wet tissue in 500 µL – 1 mL of chilled 1X Cell Extration Buffer PTR. For lower amounts of tissue adjust volumes accordingly. Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extration Buffer PTR.

Plate Preparation

The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
For each assay performed, a minimum of two wells must be used as the zero control.
For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
Differences in well absorbance or "edge effects" have not been observed with this assay.

Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.
We recommend that you assay all standards, controls and samples in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.
3. Add 50 µL of all sample or standard to appropriate wells.
4. Add 50 µL of the Antibody Cocktail to each well.
5. Seal the plate and incubate for 30 minutes at room temperature on a plate shaker set to 750 rpm.
6. Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 30 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.
7. Add 50 µL of prepared Lumi HRP Development Solution to each well and incubate for 1 minute in the dark on a plate shaker set to 750 rpm. Further optimization of incubation time vs signal strength can be performed if needed. Avoid introducing bubbles into the wells.
8. Measure the produced light of each well using a microplate luminometer with the following settings: 0.5-1 second/well read time in summation mode (all wavelengths). Relative light unit (RLU) readings may vary between luminometer models. It is recommended to configure instrument settings according to the manufacturer's specifications. Note: Relative light unit (RLU) values may change over the course of the 15-minute reading window.
9. Analyze the data as described below.

Mode:	Luminescence
Instrument settings:	Endpoint
Detection Mode:	All wavelengths
Read Time:	0.5-1 sec
Read:	Top

Note For microplate readers with Pre-Read Optimization option, the Read Height as well as Microplate Optimization is recommended before the first read.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
https://www.abcam.com/en-us/technical-resources/guides/elisa-guide

Technical Support

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For all technical or commercial enquiries please go to:
https://www.abcam.com/en-us/contact-us
https://www.abcam.cn/contact-us (China)
https://www.abcam.co.jp/contact-us (Japan)

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Additional information

ASSAY SPECIFICITY
This it is designed for the quantification fo human AGT.

Native signal was detected in serum, plasma (citrate), plasma (EDTA), plasma (heparin), and tissue extract.

Spiked protein experiments were used to validate cell culture supernatant sample types.

Urine, saliva, milk, and cerebrospinal fluid samples have not been tested with this kit.

This kit is not compatible with cell culture media containing bovine serum.

CROSS REACTIVITY
5,000 pg/mL of recombinant human ACE2 and 5,000 pg/mL of recombinant human TMPRSS2 were tested for cross reactivity. No cross reactivity was observed.

INTERFERENCE
5,000 pg/mL of recombinant human ACE2 and 5,000 pg/mL of recombinant human TMPRSS2 were tested for interference with 5,000 pg/mL of recombinant human AGT. No interference was observed.

SPECIES REACTIVITY
Other species reactivity was determined by measuring 1:20,000 serum samples of various species, interpolating the protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in human serum assayed at the same dilution.

Reactivity < 3% swas determined for the following species: Mouse, Rat, Cow, and Monkey.

Other species reactivity not determined.

CALCULATION
- Preconfigured protocols are available when using SoftMax Pro software from Molecular Devices.
- Calculate the average chemiluminescence value for the blank control (zero) standards. Subtract the average blank control standard chemiluminescence value from all other chemiluminescence values.
- Create a standard curve by plotting the average blank control subtracted chemiluminescence value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.
- Note: Most chemiluminescence reader software or graphing software will plot these values and fit a curve to the data. A four-parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4-parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.
- Determine the concentration of the target protein in the sample by interpolating the blank control subtracted chemiluminescence values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.
- Samples generating chemiluminescence values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at chemiluminescence values less than that of the lowest standard should be retested in a less dilute form.

TYPICAL DATA
Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed

Standard Curve Measurements			
Concentration (pg/mL)	RLU		Mean RLU
	1	2	
0	2,313	2,313	2,313
49.38	3,894	3,813	3,854
148.15	8,475	9,552	9,014
444.44	23,203	22,431	22,817
1,333.33	84,091	80,370	82,231
4,000	302,160	300,290	301,225
12,000	1,097,600	1,070,500	1,084,050

Table 1. Example of human AGT standard curve in Sample Diluent NS. The AGT standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

Standard Curve Measurements			
Concentration (pg/mL)	RLU		Mean RLU
	1	2	
0	2,409	2,409	2,409
49.38	4,326	5,194	4,760
148.15	8,926	8,836	8,881
444.44	24,370	24,043	24,207
1,333.33	77,794	79,700	78,747
4,000	270,640	276,470	273,555
12,000	1,007,300	997,010	1,002,155

Table 2. Example of human AGT standard curve in 1X Cell Extraction Buffer PTR. The AGT standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

TYPICAL SAMPLE VALUES

Sensitivity:

The minimal detectable dose (MDD) was determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentration.

Sample Diluent Buffer	N=	Minimal Detectable Dose
Sample Diluent NS	16	19.3 pg/mL
1X Cell Extraction Buffer PTR	16	17.1 pg/mL

Recovery

Three concentrations of AGT were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
1:40,000 Serum	107	104 - 110
1:40,000 Plasma - Citrate	88	85 - 94
1:40,000 Plasma - EDTA	104	98 - 109
1:40,000 Plasma - Heparin	105	91 - 117
100% Cell Culture Media*	97	83 - 110
6.25 µg/mL Lung Tissue Extract	88	84 - 91

*Media is RPMI 1640 without fetal calf serum. This kit is not compatible with media containing bovine serum.

Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native AGT was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	1:20,000 Human Serum	1:20,000 Human Plasma (Citrate)	1:20,000 Human Plasma (EDTA)	1:20,000 Human Plasma (Heparin)
Undiluted	pg/mL	2421.15	2625.54	2183.02	2470.71
	% Expected value	100	100	100	100
2	pg/mL	1181.95	1278.36	1038.38	1202.24
	% Expected value	98	97	95	97
4	pg/mL	564.32	596.76	495.72	575.17
	% Expected value	93	91	91	93
8	pg/mL	285.62	289.22	235.19	275.22
	% Expected value	94	88	86	89
16	pg/mL	123.38	134.67	NL	130.04
	% Expected value	82	82	NL	84

NL – Non-Linear

Recombinant AGT was spiked into the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	100% Cell Culture Media
Undiluted	pg/mL	4280.77
	% Expected value	100
2	pg/mL	1995.83
	% Expected value	93
4	pg/mL	1007.99
	% Expected value	94
8	pg/mL	509.23
	% Expected value	95
16	pg/mL	251.82
	% Expected value	94

Native AGT was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in 1X Cell Extraction Buffer PTR.

Dilution Factor	Interpolated value	25 µg/mL Human Lung Tissue Extract
Undiluted	pg/mL	4216.21
	% Expected value	100
2	pg/mL	2137.42
	% Expected value	101
4	pg/mL	1049.43
	% Expected value	100
8	pg/mL	504.34
	% Expected value	96
16	pg/mL	234.13
	% Expected value	89

Precision

Mean coefficient of variations of interpolated values of AGT from three concentrations of recombinant protein within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	3.1	6.5

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
https://www.abcam.com/en-us/technical-resources/guides/elisa-guide

Technical Support

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