# HUMAN ANGIOTENSINOGEN (AGT) ELISA KIT

**FOR THE QUANTITATIVE DETERMINATION OF HUMAN AMGIOTENSINOGEN CONCENTRATIONS IN SERUM AND PLASMA**

**ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.**

**FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

**PRODUCT INFORMATION:**

**THIS KIT IS FOR ONE TIME USE ONLY.**

| **ELISA NAME** | **HUMAN ANGIOTENSINOGEN ELISA** |
|---|---|
| **Catalog No.** | SK00522-06 |
| **Formulation** | 96 T |
| **Lot No.** | |
| **Standard range** | 0.625 – 40 ng/mL |
| **Sensitivity** | 150 pg/mL |
| **Sample Volume** | 100 µL |
| **Dilution Factor** | 500 ~1000 for serum **(Optimal dilutions should be determined by each laboratory for each application)** |
| **Sample Type** | Serum, Plasma |
| **Specificity** | Human Angiotensinogen |
| **Calibration** | Human AGT (HEK293) |
| **Intra-assay Precision** | 4 - 6% |
| **Inter-assay Precision** | 8 - 10% |
| **Storage** | 2 – 8° C for 1 month. See page 2~3 for detail. |
| **This kit contains sufficient materials to run approximately 35 samples duplicated provided that assay is run according to protocol.** | |

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## DESCRIPTION

This Human Angiotensinogen (AGT) ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human Angiotensinogen from serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant Angiotensinogen and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural human Angiotensinogen samples.

## ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for Angiotensinogen. The capture antibody can bind to the human Angiotensinogen in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against Angiotensinogen is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of human Angiotensinogen bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

## PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix reagents with those from other lots or sources.
_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_Each laboratory must determine the optimal dilution factors for the samples being assayed.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

## COMPONENTS PROVIDED

| DESCRIPTION | CODE | QUANTITY |
|---|---|---|
| **Angiotensinogen Microplate** – 96 well microplate coated with an antibody specific for Angiotensinogen. | **522-06-01** | **1 plate** |
| **Angiotensinogen Standard** – refer to lot of lyophilized recombinant Angiotensinogen. | **522-06-02** | **1 vial** |
| **Detection Antibody Concentrate** – refer to lot of 10-fold concentrate of lyophilized biotinylated antibody against Angiotensinogen. | **522-06-03** | **1 vial** |
| **Positive Control** – one vial of lyophilized recombinant Autotaxin. | **522-06-04** | **1 vial** |
| **Streptavidin-HRP Conjugate** – 120 µL/vial of 100-fold concentrated solution of Streptavidin-HRP conjugate. | **SAHRP** | **1 vial** |
| **Dilution Buffer** – 50 mL of buffered solution with preservative. | **DB10** | **2 bottles** |
| **Antibody & HRP Diluent Solution** – 25 mL of buffered solution with preservative. | **DB108A** | **1 bottle** |
| **Wash Buffer** – 50 mL of 10-fold concentrated buffered surfactant with preservative. | **WB01** | **1 bottle** |
| **TMB Substrate Solution** – 11 mL of TMB substrate solution. | **TMB01** | **1 bottle** |
| **Stop Solution** – 11 mL of 0.5M HCl. | **S-STOP** | **1 bottle** |
| **Plate Sealer** | **EAPS** | **1 piece** |
| **Plastic Pouch** | **P01** | **1 piece** |

## STORAGE

**Unopened Kit:** Store at 2 – 8° C for up to 1 month. For longer storage up to 12 months, unopened Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer and Antibody & HRP Diluent Solution should be stored at -20° C. **Streptavidin-HRP Conjugate** and TMB Substrate

Solution should be stored only at 2 – 8 ˚C.Do not use kit past expiration date.

## ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

## PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

## SAMPLE COLLECTION AND STORAGE

**Serum** – Use a serum separator tube (SST). Allow blood to clot for 30 minutes. Centrifuge at 1000 x g for 15 minutes and collect serum. Assay samples immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

**Plasma** - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

## SAMPLE PREPARATION

Serum samples may require 500-1000 fold dilutions. A suggested 50-fold dilution is 5 µL sample + 245 µL Dilution Buffer. A final 500-fold dilution is 25 µL of 50-fold diluted sample solution + 225 µL Dilution Buffer. A final 1000-fold dilution is 12.5 µL of 50-fold diluted sample solution + 237.5 µL Dilution Buffer. **Optimal dilutions should be determined by each laboratory for each application with a pretest. Use polypropylene test tubes.**

## REAGENT PREPARATION

**Bring all reagents to room temperature before use.**

**Wash Buffer** – If crystals have formed in the concentrate, warm bottle in a water bath until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into 450 mL distilled or deionized water to make 500 mL of 1x Wash Buffer.

**Angiotensinogen Standard** – Reconstitute the Angiotensinogen standard with refer to lot of Dilution Buffer. The concentration of the reconstituted stock solution is 40 ng/mL. Allow the stock standard to sit for at least 15 minutes with gentle agitation until completely dissolved prior to making standard dilutions (see below). Mix each tube thoroughly before the next transfer.

| TUBE | STANDARD | DILUTION BUFFER | CONCENTRATION |
|---|---|---|---|
| Stock | powder | Refer to lot | 40 ng/mL |
| # 1 | 80µL of stock | 240µL | 10 ng/mL |
| # 2 | 80µL of 1 | 240µL | 2.5 ng/mL |
| # 3 | 80µL of 2 | 240µL | 0.625 ng/mL |

**Positive Control** - Reconstitute the Positive Control with refer to lot of Dilution Buffer.

**Detection Antibody Concentrate -** Reconstitute the Detection Antibody Concentrate with refer to lot of Dilution Buffer to produce a 10-fold concentrated stock solution. Allow the concentrated solution to sit for at least 5 minutes until completely dissolved. Pipette 9.45 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

**Streptavidin-HRP Conjugate -** Pipette 11.88 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution. **Protect from light**. **DO NOT FREEZE.**

## ELISA PROTOCOL

**Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.**

1. Prepare all reagents, standard dilutions, positive control and samples as directed previously.

2. Add 100 µL per well of **Dilution Buffer** to Blank wells.
3. Add 100 µL per well of **Standard Dilutions** (in reverse order of serial dilution from #6 - S), **samples**, or **positive control**. Cover with plate sealer and incubate for 2 hours on microplate shaker at room temperature.
4. Aspirate and wash each well with 300 µL of **1x Wash Buffer** four times. After the last wash, aspirate any remaining 1x Wash Buffer, invert the plate and blot against clean paper towel(s).
5. Add 100 µL per well of **Detection Antibody working solution**. Cover with plate sealer and incubate for 2 hours on microplate shaker at room temperature.
6. Repeat the aspiration and wash as in step 4.
7. Add 100 µL per well of **Streptavidin-HRP Conjugate working solution**. Cover with plate sealer and incubate for 45 minutes on microplate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration and wash as in step 4.
9. Add 100 µL per well of **Substrate Solution**. Incubate for refer to lot on microplate shaker at room temperature. **Protect from light.**
10. Add 100 µL per well of **Stop Solution**. The color in the wells should change from blue to yellow. If the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
11. Read plate using a microplate reader set to 450 nm.

## CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

## SPECIFICITY

| PROTEIN | CROSS-REACTIVITY |
|---|---|
| Human Angiotensinogen | 100 |
| Human Angiotensin II | 0 |
| Human Angiotensin I | 0 |
| Human DPPIV | 0 |

## TYPICAL DATA

This standard curve is for demonstration only. A new standard curve should be made for each set of samples assayed.

| AUTOTAXIN (NG/ML) | CORRECTED (450NM) |
|---|---|
| Blank | 0 (0.056) |
| 0.625 | 0.021 |
| 2.5 | 0.109 |
| 10 | 0.319 |
| 40 | 1.089 |

## SUMMARY OF ASSAY PROCEDURE

**PREPARE REAGENTS, SAMPLES AND STANDARD DILUTIONS**

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Add 100 µL per well of standard dilutions, samples and positive control. Cover with plate sealer and incubate 2 hours on microplate shaker at RT.

↓

Aspirate and wash 4 times.

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Add 100 µL per well of Detection Antibody working solution. Cover with plate sealer and incubate 2 hours on microplate shaker at RT.

↓

Aspirate and wash 4 times.

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| |
|---|
| Add 100 µL per well of Streptavidin-HRP Conjugate working solution. Cover with plate sealer and incubate 40 minutes on microplate shaker at RT. **Protect from light**. |
| ⇩ |
| Aspirate and wash 4 times. |
| ⇩ |
| Add 100 µL per well of Substrate Solution. Incubate refer to lot on microplate shaker at RT. **Protect from light**. |
| ⇩ |
| Add 100 µL per well of Stop Solution. Read at 450 nm. |