Human Angiotensin II ELISA Kit

Enzyme Immunoassay for the quantification of human Angiotensin II (Ang II) in cell supernatants, serum, plasma and tissue homogenates.

Catalog number: ARG80961

Package: 96 wells

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INTRODUCTION

Angiotensinogen is an essential component of the renin-angiotensin system (RAS), a potent regulator of blood pressure, body fluid and electrolyte homeostasis.

Angiotensin-2: Angiotensin II (Ang II) acts directly on vascular smooth muscle as a potent vasoconstrictor, affects cardiac contractility and heart rate through its action on the sympathetic nervous system, and alters renal sodium and water absorption through its ability to stimulate the zona glomerulosa cells of the adrenal cortex to synthesize and secrete aldosterone.

Angiotensin-3: stimulates aldosterone release.

Angiotensin 1-7: is a ligand for the G-protein coupled receptor MAS1. Has vasodilator and antidiuretic effects. Has an antithrombotic effect that involves MAS1-mediated release of nitric oxide from platelets.[UniProt]

PRINCIPLE OF THE ASSAY

This is an Enzyme Immunoassay for the quantification Angiotensin II (Ang II) in human cell supernatants, serum, plasma and tissue homogenates. samples. This assay employs the competitive quantitative enzyme immunoassay technique.

An Angiotensin II (Ang II) antibody has been pre-coated onto a microtiter plate. The primary antibodies on the plate will be competitively bound by biotinylated- Angiotensin II (Ang II) and Angiotensin II (Ang II) in standards or targeted Angiotensin II (Ang II) in samples. The wells are washed and then incubated with Streptavidin-HRP reagent. The biotinylated Angiotensin II (Ang II) interacts with streptavidin-horseradish peroxidase to form a complex. After

washing away any unbound Streptavidin-HRP reagent, a substrate solution (TMB) is added to the wells and color develops in inverse-proportion to the amount of Angiotensin II (Ang II) present in the samples. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450nm±2 nm. The concentration of Angiotensin II (Ang II) in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon receipt, store Standard and Biotinylated Human Ang II at-20°C or -80°C (-80°C is recommended) and store all other kit components at 4°C at all times. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	12 x 8 wells	4°C
Standard (Human Ang II)	2 X 10 ng/Vial (Lyophilized)	-20°C or -80°C
Biotinylated Human Ang II	1 vial (Lyophilized)	-20°C or -80°C
40X Streptavidin-HRP conjugate	300 µl	4°C
Diluent Buffer	3 X 12 ml	4°C
20X Wash buffer	25 ml	4°C
Chromogen solution A	6 ml	4°C (Protect from light)
Chromogen solution B	6 ml	4°C (Protect from light)
STOP solution	6 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon receipt, store Standard and Biotinylated Human Ang II at-20°C or-80°C (-80°C is recommended) and store all other kit components at 4°C at all times. Avoid repeated freeze-thaw cycles.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming. Briefly spin down the reagents to bring down all components to the bottom of tubes before opening.
- If crystals are observed in the 20X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards and samples be assayed in duplicates.
- Stop Solution should be added in the same order of the TMB substrate solution.
- Do not let strips dry, as this will inactivate active components in wells.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatants - Remove particulates by centrifugation for 10 min at 3000 x g at 4°C. Collect the supernatants and assay immediately or aliquot & store samples at ≤-20°C for up to 3 months. Avoid repeated freeze-thaw cycles.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C for up to 3 months. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20°C for up to 3 months. Avoid repeated freeze-thaw cycles.

Tissue lysate- Extract tissue samples with ice-cold lysis buffer. Lysis samples by homogenization or by sonication on ice. Then centrifuge at 14,000 rpm for 20 min at 4°C. Collect the supernatant and measure the protein concentration. Aliquot and store remaining extract at-80°C for up to 3 months. Avoid repeated freeze-thaw cycles.

Note:

a) Do not use haemolytic, icteric or lipaemic specimens.
b) Samples containing sodium azide should not be used in the assay.
c) Since the concentrations in cell culture supernatant is influenced by the factors including cell viability, cell number and also sampling time, samples from cell culture supernatant may detect a concentration lower than the lowest standard.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer. (E.g. 25 ml of 20X Wash buffer + 475 ml of distilled water) The diluted Wash buffer is stable for 4 weeks at 2°C to 8°C.
- Samples: Serum and plasma samples have to be diluted at 1000X by Diluent Buffer before assay. And assay the diluted samples immediately, the diluted samples should be assayed within 15 mins after dilution. (Add 1 part of sample into 4 parts of Diluent Buffer.) If samples give OD readings higher than the highest standard, please dilute samples with Diluent Buffer and repeat the assay. For the calculation of the concentrations this dilution factor has to be taken into account. (It is recommended to do pre-test to determine the suitable dilution factor).
- Biotinylated Human Ang II: Prepared within 2 hours prior to use. Reconstitute the Biotinylated Human Ang II vial with 1 ml of Diluent Buffer to yield a biotinylated Human Ang II stock solution. Allow it to sit for 5-10 minutes to completely dissolve, mix well. Then dilute the biotinylated Human Ang II stock 1:5 with Diluent Buffer to yield biotinylated Human Ang II working solution, mix thoroughly. (e.g. 300 µL of biotinylated Human Ang II stock+ 1.5 ml of Diluent Buffer) Keep the diluted solution at 4°C before use. For long-term storage, it is recommended to aliquot & store the stock solution at -70°C for up to 6 months. Avoid repeated freeze-thaw cycles.
- 1X Streptavidin-HRP conjugate: Prepared within 1 hours prior to use. Centrifuge 40X Streptavidin-HRP conjugate briefly and dilute 40X Streptavidin-HRP concentrate solution into Diluent Buffer to yield 1X

Streptavidin-HRP Solution buffer. Keep diluted 1X Streptavidin-HRP Solution in dark before use. (E.g. 100 µL 40X Streptavidin-HRP conjugate + 3.9 ml of Diluent Buffer) Store the rest of 40X Streptavidin-HRP conjugate at 4°C.

- **Standards:** Standard solution should be prepared within 2 hours prior to the experiment. Reconstitute the standard with **1 ml** of **Diluent Buffer** to yield a stock concentration of **10 ng/ml**. Allow the stock standard to sit for at least 10 minutes with gentle agitation to make sure the standard is dissolved completely before making serial dilutions. The stock standard can be aliquoted & stored at -70°C for up to 6 months. Avoid repeated freeze-thaw cycles. The Diluent Buffer serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted with Diluent Buffer as according to the suggested concentration below: **10000 pg/ml**, **1000 pg/ml**, **100 pg/ml**, **10 pg/ml**, **1 pg/ml.**

The example of the dilution of standards

Standard No.	Standard Conc. (ng/ml)	Diluent Buffer (µl)	Standard (µl)
S1 (Stock)	10000	-	-
S2	1000	450	50 µl of S1
S3	100	450	50 µl of S2
S4	10	450	50 µl of S3
S5	1	450	50 µl of S4
S0 (Total binding)	0	450	0

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards and samples should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **50 µl of Diluent Buffer** as Total Binding (S0, zero standard). Two empty wells should be left as blank.
3. Add **50 µl** of **standards** (add from S5 to S1) **and (diluted) samples** in duplicate into the appropriate wells.
4. Immediately, add **50 µl** of **Biotinylated Human Ang II** to each well **except the Blank wells**. Gently tap the plate to ensure thorough mixing.
5. Cover the plate and incubate the plate for **2 hour at room temperature.**
6. Aspirate each well and **wash**, repeating the process 4 times for a **total 5 washes**. Wash by filling each well with **1× Wash Buffer (350 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
7. Add **100 µl** of **1X Streptavidin-HRP conjugate** to each well (except the Blank wells.), gently tap the plate to mix well. Cover wells and incubate for **30 minutes at RT**.
8. Aspirate each well and **wash as step 6.**
9. Add **50 µl** of **Chromogen solution A** and **50 µl** of **Chromogen solution B** to each well (including the Blank wells). Cover wells and incubate for **10-25 minutes at RT.** Protect from light. (Note: The incubation time is for

reference only, the optimal incubation time should be determined by end user. When the shades of blue color can be seen in the wells with S1 and S2 standard solutions).

10. Add 50 µl of Stop Solution to each well. The color of the solution should change from blue to yellow. Gently tap the plate to ensure thorough mixing.
11. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards and samples.

2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

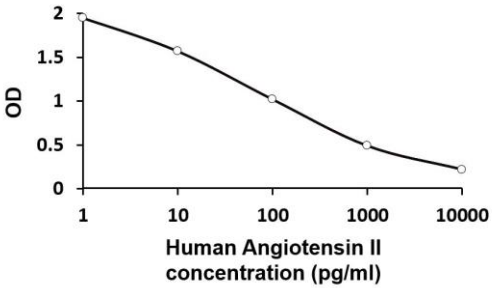
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Angiotensin 2 ranged from 1-10000 pg/ml. The mean MDD was 2 pg/ml.

Specificity

This assay recognizes Human Angiotensin II. No significant cross-reactivity or interference with Ang I and other peptide, protein.