Human Angiotensin 1-7 ELISA Kit

Enzyme Immunoassay for the quantification of human Angiotensin 1-7 in serum, plasma and cell culture supernatant

Catalog number: ARG80960

Package: 96 wells

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MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

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: 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 assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human Angiotensin 1-7 has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Angiotensin 1-7 present is bound by the immobilized antibody. A HRP-conjugated antibody specific for Angiotensin 1-7 is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a chromogenic substrate solution is added to the wells and color develops in proportion to the amount of Angiotensin 1-7 bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color

is measured at a wavelength of 450 nm ±2 nm. The concentration of Angiotensin 1-7 in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	12 wells X 8 strips	4°C
Standards A-F (Concentration: 15.6, 31.25, 62.5, 125,250,500 pg/ml)	6 vials X 0.5ml	4°C
Sample Diluent	6 ml	4°C
HRP-conjugated Antibody	10 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
Plate sealer	2	RT

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- 37 °C incubator
- 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.
- Store the kit at 2-8°C at all times. Store the unused strips in a sealed foil bag at 2-8°C.
- If crystals are observed in the 20X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- All materials should be equilibrated to room temperature (RT, 22-25°C) 20 min before use.
- Ensure complete dilution of reagents prior to use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Pipette reagents and samples into the center of each well, avoid bubbles.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Avoid cross-contamination by changing tips, using separate reservoirs for each reagent.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Stop Solution should be added in the same order of the Substrate Solution.

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 and aliquot & store samples at ≤ -20 °C (≦1 month) or -80 °C (≦6 month). 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 (≦1 month) or -80 °C (≦6 month). 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 (≦1 month) or -80 °C (≦6 month). Avoid repeated freeze-thaw cycles. Heat-treated, hemolytic samples and samples containing sodium azide are not recommended for this kit.

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: If samples give OD readings higher than the highest standard provided, please dilute samples with sample diluent 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).

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 22-25°C) 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 **Sample Diluent Buffer** in duplicate into wells (S0, blank).
3. Add **50 µl** of **standards and samples** in duplicate into wells.
4. Add **100 µl** of **HRP-conjugated antibody** to each well.
5. Mix well gently. It is important mixing well in this step. Cover the plate and incubate the plate for **1 hour at 37°C.**
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. (For Automated Washing, It is recommended that the washer be set for a soaking time of 10 seconds and shaking time of 5 seconds between each)
7. Add **50 µl** of **Chromogen solution A** and **50 µl** of **Chromogen solution B** to each well subsequently. Cover wells and incubate for **15 minutes at 37°C**. Protect from light. Note: If the blue color develops too light after 15 minutes incubation with the substrates, it may be appropriate to extend the incubation time. (Do not over-develop)
8. Add **50 µl** of **Stop Solution** to each well. Mix well.
9. 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 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.

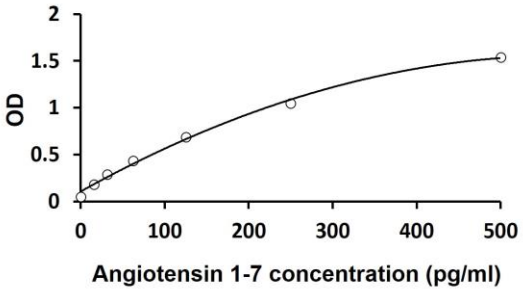
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

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 1-7 ranged from 15.6-500 pg/ml. The mean MDD was 2 pg/ml.

Specificity

This assay has high sensitivity and excellent specificity for detection of Human Angiotensin 1-7.