Human ACE ELISA Kit

Enzyme Immunoassay for the quantification of human ACE in serum, plasma and cell culture supernatant

Catalog number: ARG80958

Package 96 wells

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Angiotensin-converting enzyme (ACE) is an enzyme involved in catalyzing the conversion of angiotensin I into a physiologically active peptide angiotensin II. Angiotensin II is a potent vasopressor and aldosterone-stimulating peptide that controls blood pressure and fluid-electrolyte balance. This enzyme plays a key role in the renin-angiotensin system. Many studies have associated the presence or absence of a 287 bp Alu repeat element in this gene with the levels of circulating enzyme or cardiovascular pathophysiologies. Multiple alternatively spliced transcript variants encoding different isoforms have been identified, and two most abundant spliced variants encode the somatic form and the testicular form, respectively, that are equally active. [provided by RefSeq, May 2010]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human ACE has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any ACE present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for human ACE is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of ACE 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 450nm ±2nm. The concentration of human ACE 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	96 well	4°C
Standards	2 X 8 ng/vial	4°C, Lyophilized
Sample Diluent	2 X 12 ml	4°C
Biotinylated ACE antibody (60X)	1 vial	4°C
Antibody Diluent	12 ml (Ready to use)	4°C
Streptavidin-HRP concentrate (40X)	1 vial	4°C
Streptavidin-HRP Diluent	12 ml (Ready to use)	4°C
20X Wash buffer	25 ml	4°C
Chromogen Solution A	6 ml	4°C
Chromogen Solution B	6 ml	4°C (Protect from light)
STOP solution	12 ml (Ready to use)	4°C
Plate sealer	2	RT

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.
- Store the kit at 4°C at all times. Biotinylated ACE antibody (60X) is stable at 4°C for up to 1 month after opening. For long-term storage, aliquot and store antibody at -20°C.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- 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.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Do not let strips dry, as this will inactivate active components in wells.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

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. 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. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using heparin or EDTA 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. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **1X Wash buffer**: Dilute **20X Wash buffer** into **distilled water** to yield 1X Wash buffer.
- **Biotinylated ACE antibody**: Dilute **60X** concentrated Biotinylated ACE antibody in Antibody Diluent buffer. The working solution should be prepared no more than 2 hours prior to the experiment so it should be prepared fresh. (Note: the volume of working solution per well is 0.1 ml)
- **Streptavidin-HRP working solution**: The Streptavidin-HRP working solution should be prepared no more than 1 hour prior to use. Streptavidin-HRP should be diluted **40X** with Streptavidin-HRP Diluent buffer and mixed thoroughly. (Note: the volume of working solution per well is 0.1 ml)
- **TMB Substrate solution**: Mix **equal volume** of **Chromogen Solution A and**

Chromogen Solution B, within 10 min before use. Prepare reagent freshly, and only prepare the needed volume for once. Protect from light.

- **Sample**: If the initial assay found samples contain ACE higher than the highest standard, the samples can be diluted with Sample diluent and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account. The sample must be well mixed with the diluents buffer before assay.

  (It is recommended to do pre-test to determine the suitable dilution factor).
- **Standards**: Reconstitute lyophilized vial **(8 ng/vial)** with **1000 µl** of Sample Diluent buffer to generate **8000 pg/ml** stock standard. Make sure the standard is dissolved completely before making serial dilutions. The Sample Diluent buffer serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted as according to the suggested concentration below: **8000 pg/ml**, **4000 pg/ml**, **2000 pg/ml**, **1000 pg/ml**, **500 pg/ml**, **250 pg/ml**, and **125 pg/ml**. The standard solutions are best used within 2 hours.

Dilute ACE standard as according to the table below:

Standard	ACE Conc. (pg/ml)	µl of Sample diluent	µl of standard
S7	8000 pg/ml	0	1000 (8000 pg/ml Stock)
S6	4000 pg/ml	500	500 (S7)
S5	2000 pg/ml	500	500 (S6)
S4	1000 pg/ml	500	500 (S5)
S3	500 pg/ml	500	500 (S4)
S2	250 pg/ml	500	500 (S3)
S1	125 pg/ml	500	500 (S2)
S0 (blank)	0	500	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 **100 µl** of sample diluent buffer in duplicate into wells (blank).
3. Add **100 µl** of **standards and samples** in duplicate into wells.
4. Cover with plate sealer and incubate the plate for **2 hours at room temperature** (RT).
5. Aspirate each well and wash, repeating the process 2 times for a **total 3 washes**. Wash by filling each well with **1× Wash Buffer (300 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.
6. Add **100 µl** of **diluted Biotinylated ACE antibody** to each well. Cover wells and incubate for **2 hours at RT**.
7. Aspirate each well and **wash as step 5**.
8. Add **100 µl** of **Streptavidin-HRP working solution** in each well. Cover wells and incubate for **30 minutes at RT**. Avoid exposure to direct light.
9. Aspirate each well and **wash as step 5**.
10. Add **100 µl** of **Mixed TMB Substrate** to each well. Incubate for **5-30 minutes at room temperature** or until a gradient develops and you see visible color in the 2nd lowest concentration well in dark.

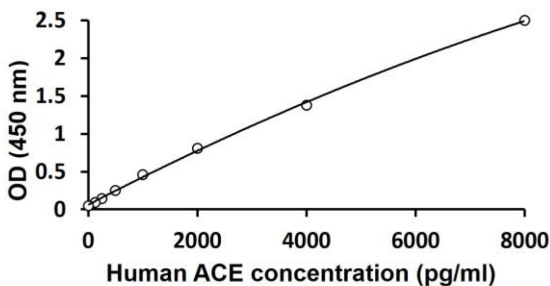
11. Add **50 µl** of **Stop Solution** to each well. Mix well.
12. Read the OD with a microplate reader at **450 nm** immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards and amples.
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 human ACE ranged from 125-8000 pg/ml. The mean MDD was 100 pg/ml.

Specificity

This assay recognizes both natural and recombinant human ACE.

Recovery

89 – 108 %

Intra-assay and Inter-assay precision

The CV values of both intra and inter precision fall below 9%.