ACE (Human) ELISA Kit

Catalog Number KA0432

96 assays

Version: 06

Intended for research use only

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Introduction

Intended Use

For quantitive detection of human ACE in cell culture supernates, serum, plasma (heparin) and saliva.

Background

Angiotensin-converting enzyme (ACE) is a zinc-containing dipeptidyl carboxypeptidase widely distributed in mammalian tissues and is thought to play a critical role in blood pressure regulation. The predicted protein is identical, from residue 37 to its C terminus, to the second half or C-terminal domain of the endothelial ACE sequence. The protein sequence inferred consists of a 732-residue preprotein including a 31-residue signal peptide. The mature polypeptide has a molecular weight of 80,073.[1] Although ACE has been studied primarily in the context of its role in blood pressure regulation, this widely distributed enzyme has many other physiological functions. The ACE gene encodes two isozymes. The somatic isozyme is expressed in many tissues, including vascular endothelial cells, renal epithelial cells, and testicular Leydig cells, whereas the testicular or germinal angiotensin-converting enzyme is expressed only in sperm.[2] The standard product used in this kit is recombinant human ACE, consisting of 30-1261 amino acids with the molecular mass of 120KDa.

Principle of the Assay

ACE (Human) ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. A monoclonal antibody from mouse specific for ACE has been precoated onto 96-well plates. Standards (NSO, L30-L1261) and test samples are added to the wells, a biotinylated detection polyclonal antibody from goat specific for ACE is added subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the human ACE amount of sample captured in plate.

General Information

Materials Supplied

List of component

Component	Amount
One 96-well plate precoated with anti- human ACE antibody	96 (8x12) wells
Lyophilized recombinant human ACE standard	10 ng/tube x 2
Biotinylated anti- human ACE antibody, dilution 1:100	130 µL
Avidin-Biotin-Peroxidase Complex (ABC), dilution 1:100	130 µL
Sample diluent buffer	30 mL
Antibody diluent buffer	12 mL
ABC diluent buffer	12 mL
TMB color developing agent	10 mL
TMB stop solution	10 mL

Storage Instruction

Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles.

Materials Required but Not Supplied

- ✓ Microplate reader in standard size.
- ✓ Automated plate washer.
- ✓ Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
- ✓ Clean tubes and Eppendorf tubes.
- ✓ Washing buffer (neutral PBS or TBS).
    - Preparation of 0.01M TBS:
      Add 1.2g Tris, 8.5g NaCl; 450 µL of purified acetic acid or 700 µL of concentrated hydrochloric acid to 1000 mL H_2O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1 L.
    - Preparation of 0.01 M PBS:
      Add 8.5 g sodium chloride, 1.4 g Na_2HPO_4 and 0.2 g NaH_2PO_4 to 1000 mL distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

Precautions for Use

- ✓ To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
- ✓ The TMB Color Developing agent is colorless and transparent before using, contact us freely if it is not the case.
- ✓ Before using the Kit, spin tubes and bring down all components to bottom of tubes.
- ✓ Duplicate well assay was recommended for both standard and sample testing.
- ✓ Don't let 96-well plate dry, for dry plate will inactivate active components on plate.
- ✓ Don't reuse tips and tubes to avoid cross contamination.
- ✓ To avoid to use the reagents from different batches together.
- ✓ In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution will be pre-warmed in 37°C for 30 min before using.

Assay Protocol

Reagent Preparation

- Reconstitution of the human ACE standard: ACE standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of ACE standard (10 ng per tube) are included in each kit. Use one tube for each experiment.
  - ✓ 10,000 pg/mL of human ACE standard solution: Add 1 mL sample diluent buffer into one tube, keep the tube at room temperature for 10 min and mix thoroughly.
  - ✓ 5000 pg/mL→156 pg/mL of human ACE standard solutions: Label 6 Eppendorf tubes with 5000 pg/mL, 2500 pg/mL, 1250 pg/mL, 625 pg/mL, 313 pg/mL, 156 pg/mL, respectively. Aliquot 0.3 mL of the sample diluent buffer into each tube. Add 0.3 mL of the above 10,000 pg/mL ACE standard solution into 1st tube and mix. Transfer 0.3 mL from 1st tube to 2nd tube and mix. Transfer 0.3 mL from 2nd tube to 3rd tube and mix, and so on.

  Note: The standard solutions are best used within 2 hours. The 10ng/mL standard solution may be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

- Preparation of biotinylated anti-human ACE antibody working solution: The solution should be prepared no more than 2 hours prior to the experiment.
  - ✓ The total volume should be: 0.1 mL/well x (the number of wells). (Allowing 0.1-0.2 mL more than total volume).
  - ✓ Biotinylated anti-human ACE antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly (i.e. Add 1 µL Biotinylated anti-human ACE antibody to 99 µL antibody diluent buffer.)

- Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution: The solution should be prepared no more than 1 hour prior to the experiment.
  - ✓ The total volume should be: 0.1 mL/well x (the number of wells). (Allowing 0.1-0.2 mL more than total volume)
  - ✓ Avidin- Biotin-Peroxidase Complex (ABC) should be diluted in 1:100 with the ABC dilution buffer and mixed thoroughly. (i.e. Add 1 µL ABC to 99 µL ABC diluent buffer.)

Sample Preparation

✓ Sample Preparation and Storage

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

- Cell culture supernate: Remove particulates by centrifugation, assay immediately or aliquot and store at -20°C.
- Serum: Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1000 X g for 15 min. Analyze the serum immediately or aliquot and store samples at -20°C.
- Plasma: Collect plasma using heparin as an anticoagulant. Centrifuge for 15 min at 1500 x g within 30 min of collection. Assay immediately or aliquot and store samples at -20°C.
- Saliva: Collect saliva using a collection device without any protein binding or filtering capabilities such as a Salivette or aliquot and store samples at -20°C.

✓ Sample Dilution Guideline

The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve. Dilute the sample using the provided diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice. The sample must be well mixed with the diluents buffer.

- High target protein concentration (100-1000 ng/mL). The working dilution is 1:100. i.e. Add 1 µL sample into 99 µL sample diluent buffer.
- Medium target protein concentration (10-100 ng/mL). The working dilution is 1:10. i.e. Add 10 µL sample into 90 µL sample diluent buffer.
- Low target protein concentration (156-10,000 pg/mL). The working dilution is 1:2. i.e. Add 50 µL sample to 50 µL sample diluent buffer.
- Very Low target protein concentration (≤156 pg/mL). No dilution necessary, or the working dilution is 1:2.

Assay Procedure

The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 min before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard ACE detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of ACE amount in samples.

1. Aliquot 0.1 mL per well of the 10,000 pg/mL, 5000 pg/mL, 2500 pg/mL, 1250 pg/mL, 625 pg/mL, 313 pg/mL, 156 pg/mL human ACE standard solutions into the precoated 96-well plate. Add 0.1mL of the sample diluent buffer into the control well (Zero well). Add 0.1 mL of each properly diluted sample of human cell culture supernatants, serum, plasma (heparin) and saliva to each empty well. See “Sample

Dilution Guideline" above for details. We recommend that each human ACE standard solution and each sample is measured in duplicate.

2. Seal the plate with the cover and incubate at 37°C for 90 min.
3. Remove the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.
4. Add 0.1 mL of biotinylated anti-human ACE antibody working solution into each well and incubate the plate at 37°C for 60 min.
5. Wash the plate 3 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (Plate Washing Method: Discard the solution in the plate without touching the side walls. Blot the plat onto paper towels or other absorbent material. Soak each well with at least 0.3 mL PBS or TBS buffer for 1~2 minutes. Repeat this process two additional times for a total of THREE washes. *Note: For automated washing, aspirate all wells and wash THREE times with PBS or TBS buffer, overfilling wells with PBS or TBS buffer. Blot the plate onto paper towels or other absorbent material.)*
6. Add 0.1mL of prepared ABC working solution into each well and incubate the plate at 37°C for 30 min.
7. Wash plate 5 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (See Step 5 for plate washing method)
8. Add 90 µL of prepared TMB color developing agent into each well and incubate plate at 37°C in dark for 20-25 min (*Note: For reference only, the optimal incubation time should be determined by end user. And the shades of blue can be seen in the wells with the four most concentrated human ACE standard solutions; the other wells show no obvious color*).
9. Add 0.1 mL of prepared TMB stop solution into each well. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450nm in a microplate reader within 30 min after adding the stop solution.

✓ Summary

1. Add samples and standards and incubate the plate at 37°C for 90 min. Do not wash.
2. Add biotinylated antibodies and incubate the plate at 37°C for 60 min. Wash plate 3 times with 0.01 M TBS.
3. Add ABC working solution and incubate the plate at 37°C for 30 min. Wash plate 5 times with 0.01 M TBS.
4. Add TMB color developing agent and incubate the plate at 37°C in dark for 20-25 min.
5. Add TMB stop solution and read.

Data Analysis

Calculation of Results

For calculation, (the relative $O.D._{450}$) = (the $O.D._{450}$ of each well) – (the $O.D._{450}$ of Zero well). The standard curve can be plotted as the relative $O.D._{450}$ of each standard solution (Y) vs. the respective concentration of the standard solution (X). The human ACE concentration of the samples can be interpolated from the standard curve. *Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.*

- Typical result
  (TMB reaction incubate at 37°C for 20 min)

Concentration (pg/mL)	0.0	156	312	625	1250	2500	5000	10,000
O.D	0.048	0.123	0.198	0.282	0.573	0.899	1.672	2.542

Performance Characteristics

✓ Range
156 pg/mL-10,000 pg/mL

✓ Sensitivity
< 5 pg/mL

✓ Specificity
Natural and recombinant human ACE.

✓ Cross-reactivity
No detectable cross-reactivity with other relevant proteins

Resources

References

1. Ehlers, M. R. W.; Fox, E. A.; Strydom, D. J.; Riordan, J. F. Molecular cloning of human testicular angiotensin-converting enzyme: the testis isozyme is identical to the C-terminal half of endothelial angiotensin-converting enzyme. Proc. Nat. Acad. Sci. 86: 7741-7745, 1989.
2. Ramaraj, P.; Kessler, S. P.; Colmenares, C.; Sen, G. C. Selective restoration of male fertility in mice lacking angiotensin-converting enzymes by sperm-specific expression of the testicular isozyme. J. Clin. Invest. 102: 371-378, 1998.

Plate Layout

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