OriGene Technologies Inc.
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Human ACE/Cd143 ELISA Kit

Catalog Number: EA100383

Assay Principle

OriGene Human ACE Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid-phase immunoassay specially designed to measure Human ACE with a 96-well strip plate that is pre-coated with antibody specific for ACE. The detection antibody is a biotinylated antibody specific for ACE. The capture antibody is polyclonal antibody from goat and the detection antibody is polyclonal antibody from goat. The kit contains recombinant Human ACE with immunogen: Expression system for standard: NS0; Immunogen sequence: L30-L1261. The kit is analytically validated with ready-to-use reagents.

To measure Human ACE, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbounded ABC-HRP with PBS or TBS buffer and add TMB. TMB is an HRP substrate and will be catalyzed to produce a blue color product, which changes into yellow after adding the acidic stop solution. The absorbance of the yellow product at 450nm is linearly proportional to Human ACE in the sample. Read the absorbance of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of Human ACE in the sample.

Overview

Product Name	*Human ACE/Cd143 ELISA Kit*
Reactive Species	*Human*
Size	*96wells/kit, with removable strips.*
Description	*Human ACE/Cd143 ELISA kit (96 Tests). Quantitate Human ACE in cell culture supernatants, serum and plasma (heparin) and saliva. Sensitivity 5pg/ml.*
Sensitivity	*<5 pg/ml* **The sensitivity or the minimum detectable dose (MDD) is the lower limit of target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.*
Detection Range	*0.78 ng/ml – 50 ng/ml*
Storage Instructions	*Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles (Shipped with wet ice.)*
Uniprot ID	*B4DKH4*

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Technical Details

Capture/Detection Antibodies	The capture antibody is a monoclonal antibody from mouse and the detection antibody is polyclonal antibody from goat.
Specificity	Natural and recombinant Human ACE
Immunogen	Expression system for standard: NS0; Immunogen sequence: L30-L1261
Cross Reactivity	There is no detectable cross-reactivity with other relevant proteins.

Notice Before Application

Please read the following instructions before starting the experiment.

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, a pilot experiment using standards and a small number of samples is recommended.
2. Before using the kit, spin tubes to bring down all components to the bottom of the tubes.
3. Don't let the 96-well plate dry out since this will inactivate active components on the plate.
4. Don't reuse tips and tubes to avoid cross-contamination.
5. Avoid using the reagents from different batches together.

Kit Components/Materials Provided

Description	Quantity	Volume
Anti-Human ACE Pre-coated 96-well strip microplate	**1**	**12 strips of 8 wells**
Human ACE Standard	**2**	**50 ng/tube**
Human ACE Biotinylated antibody (100x)	**1**	**130 µl**
Avidin-Biotin-Peroxidase Complex (100x)	**1**	**130 µl**
Sample Diluent	**1**	**30ml**
Antibody Diluent	**1**	**12ml**
Avidin-Biotin-Peroxidase Diluent	**1**	**12ml**
Color Developing Reagent (TMB)	**1**	**10ml**
Stop Solution	**1**	**10ml**
Wash Buffer	**1**	**Powder pack for 1000ml**
Plate Sealers	**4**	**Piece**

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Required Materials That Are Not Supplied

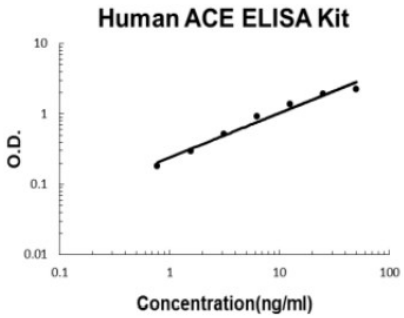
Microplate Reader capable of reading absorbance at 450nm.
Automated plate washer (optional)
1000ml of 1X wash buffer (TBS or PBS)
Pipettes and pipette tips capable of precisely dispensing 0.5 µl through 1 ml volumes of aqueous solutions.
Multichannel pipettes are recommended for large amount of samples.
Deionized or distilled water.
500ml graduated cylinders.
Test tubes for dilution.

Human ACE/Cd143 ELISA Kit (EA100383) Standard Curve Example

The Highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. value is no less than 1.0.

Concentration (pg/ml)	**0**	**0.78**	**1.56**	**3.12**	**6.25**	**12.5**	**25**	**50**
O.D.	**0.039**	**0.183**	**0.299**	**0.512**	**0.916**	**1.371**	**1.923**	**2.265**

Typical Human ACE ELISA Kit standard curve



A standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Intra/Inter Assay Variability

OriGene spend great efforts in documenting lot to lot variability and make sure our assay kits produce robust data that are reproducible.

Intra-Assay Precision (Precision within an assay): *Three samples of known concentration were tested on one plate to assess intra-assay precision.*
Inter-Assay Precision (Precision across assays): *Three samples of known concentration were tested in separate assays to assess inter-assay precision.*

	Intra-Assay Precision			*Inter-Assay Precision*		
Sample	*1*	*2*	*3*	*1*	*2*	*3*
n	*16*	*16*	*16*	*24*	*24*	*24*
Mean(pg/ml)	*1278*	*6839*	*24157*	*1358*	*6350*	*25528*
Standard deviation	*62.62*	*465.05*	*1087.06*	*86.57*	*482.6*	*1225.34*
CV(%)	*4.9 %*	*6.8 %*	*4.5 %*	*6.5 %*	*7.6 %*	*4.8 %*

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Reproducibility

To assay reproducibility, three samples with differing target protein concentrations were assayed using four different lots.

Lots	Lot1 (pg/ml)	Lot2 (pg/ml)	Lot3 (pg/ml)	Lot4 (pg/ml)	Mean (pg/ml)	Standard Deviation	CV (%)
Sample 1	1278	1310	1292	1425	1326	58.13	4.3 %
Sample 2	6839	6994	7039	6669	6885	145.23	2.1 %
Sample 3	24157	24884	22207	21600	23212	1350.66	5.8 %

*number of samples for each test n=16.

Preparation Before The Experiment

Item	Preparation
All reagents	Bring all reagents to 37°C prior to use.. The assay can also be done at room temperature however we recommend doing it at 37°C for best consistency with our QC results. Also, the TMB incubation time estimate (15-25 min) is based on incubation at 37°C.
Wash buffer	Prepare standard 1XPBS as wash buffer. Preparation of wash buffer: Add 8.5g NACl, 1.4g Na2HPO4 and 0.2g NaH2PO4 to 1000ml distilled water and adjust pH to 7.2-7,6.
Biotinylated Anti-Human ACE antibody	It is recommended to prepare this reagent immediately prior to use by diluting the Human ACE Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100 µl by adding 1 µl of Biotinylated antibody (100x) to 99 µl of Antibody Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
Avidin-Biotin-Peroxidase Complex	It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-Biotin-Peroxidase Complex (100x) 1:100 with Avidin-Biotin-Peroxidase Diluent. Prepare 100 µl by adding 1 µl of Avidin-Biotin-Peroxidase Complex (100x) to 99 µl of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
Human ACE Standard	It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Use one 10 ng of lyophilized Human ACE standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 10 ng/ml using 1ml of sample diluent. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.
Microplate	The included microplate is coated with capture antibodies and ready-to-use. It does not require additional washing or blocking. The unused well strips should be sealed and stored in the original packaging.

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Dilution of Human ACE Standard

1. Number tubes 1-8. Final Concentrations to be Tube # 1 –50,000.00 pg/ml, # 2 –25,000.00 pg/ml, # 3 – 12,500.00 pg/ml, # 4 – 6,250.00 pg/ml, # 5 – 3,125.00 pg/ml, # 6 – 1,562.50 pg/ml, # 7 – 781.25 pg/ml, # 8 – Sample Diluent serves as the zero standard (0 pg/ml).
2. For standard #1, add 1000 µl of undiluted standard stock solution to tube #1.
3. Add 300 µl of sample diluent to tubes # 2-7.
4. To generate standard # 2, add 300 µl of standard # 1 from tube # 1 to tube # 2 for a final volume of 600 µl. Mix thoroughly.
5. To generate standard # 3, add 300 µl of standard # 2 from tube # 2 to tube # 3 for a final volume of 600 µl. Mix thoroughly.
6. Continue the serial dilution for tube # 4-7

Sample Preparation and Storage

These sample collection instructions and storage conditions are intended as a general guideline and the sample stability has not been evaluated.

Sample Type	*Procedure*
Cell culture supernatants	*Clear sample of particulates by centrifugation, assay immediately or store samples at -20°C.*
Serum	*Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C.*
Plasma	*Collect plasma using heparin as an anticoagulant. Centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C.* **Note: it is important to not use anticoagulants other than the ones described above to treat plasma for other anticoagulants could block the antibody binding site.*
Saliva	Collect saliva using a collection device, aliquot and store samples at -20°C. The collection device should not have protein binding or filtering features.

Sample Dilution Guideline

The target protein concentration should be estimated and appropriate sample dilutions should be selected that the final protein concentration lies near the middle of he linear dynamic range of the assay.

It is recommended to prepare 150µl of sample for each replicate to be assayed. The samples should be diluted with sample diluent and mixed gently.

Assay Protocol

It is recommended that all reagents and materials be equilibrated to room temperature (18-25°C) prior to the experiment (see Preparation Before The Experiment if you have missed this information).

1. *Prepare all reagents and working standards as directed previously.*
2. *Remove excess microplate strips from the plate frame and seal and store them in the original packaging.*
3. *Add 100 µl of the standard, samples, or control per well. Add 100 µl of the sample diluent buffer into the control well (Zero well). At least two replicates of each standard, sample, or control is recommended.*
4. *Cover with the plate sealer provided and incubate for 120 minutes at RT (or 90 min. at 37 °C).*
5. *Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. Invert the plate on the benchtop onto a paper towel*

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and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.

6. Add 100 µl of the prepared 1x Biotinylated Anti-Human ACE antibody to each well.
7. Cover with plate sealer and incubate for 90 minutes at RT (or 60 minutes at 37°C).
8. Wash the plate 3 times with the 1x wash buffer.
   a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
   b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
   c. Repeat steps a-b 2 additional times.
9. Add 100 µl of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well. Cover with the plate sealer provided and incubate for 40 minutes at RT (or 30 minutes at 37°C).
10. Wash the plate 5 times with the 1x wash buffer.
    a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
    b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
    c. Repeat steps a-b 4 additional times.
11. Add 90 µl of Color Developing Reagent to each well. Cover with the plate sealer provided and incubate in the dark for 30 minutes at RT (or 15-25 minutes at 37°C). (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard wells, while the remaining standards remain clear.)
12. Add 100 µl of Stop Solution to each well. The color should immediately change to yellow.
13. Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450nm.

Data Analysis

Average the duplicate readings for each standard, sample, and control. Subtract the average zero standard O.D. reading.

It is recommended that a standard curve be created using computer software to generate a four parameter logistic (4-PL) curve-fit. A free program capable of generating a four parameter logistic (4-PL) curve-fit can be found online at: www.myassays.com/four-parameter-logistic-curve.assay

Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative OD against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data.

For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.

Background on ACE

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 Cterminal 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. 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. The standard product used in this kit is recombinant human ACE, consisting of 30-1261 amino acids with the molecular mass of 120KDa.