Quantikine® ELISA

Human ACE Immunoassay

Catalog Number DACE00

For the quantitative determination of human Angiotensin I Converting Enzyme (ACE) concentrations in cell culture supernates, serum, plasma, and saliva.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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MANUFACTURED AND DISTRIBUTED BY:

USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

INTRODUCTION

Angiotensin I Converting Enzyme (ACE, also known as peptidyl-dipeptidase A or CD143) is a zinc metallopeptidase important for blood pressure control and water and salt metabolism (1). It cleaves the C-terminal dipeptide from angiotensin I to produce the octapeptide angiotensin II, a potent vasopressor. It also inactivates bradykinin, a potent vasodilator, by the sequential removal of two C-terminal dipeptides. In addition to the two physiological substrates, ACE cleaves C-terminal dipeptides from various oligopeptides with a free C-terminus. Because of its location and specificity, ACE plays additional roles in immunity, reproduction and neuropeptide regulation. For example, ACE degrades Alzheimer amyloid β-peptide (Aβ), retards Aβ aggregation, deposition, fibril formation, and inhibits cytotoxicity (2). ACE inhibitors are now used clinically to treat hypertension, congestive heart failure and myocardial infarction, endothelial dysfunction and renal disease including diabetic nephropathy (3).

As a type I membrane protein subjected to shedding by secretases, ACE becomes soluble in many biological fluids, such as serum, plasma, seminal fluid, amniotic fluid and cerebrospinal fluid (1). Two ACE isoforms are transcribed from a single gene using alternative promoters (3). Somatic ACE (sACE), found in endothelial, epithelial and neuronal cells, comprises two highly similar active domains called N- and C-domains, each of which contains the HExxH consensus sequence for zinc binding. Germinal ACE (gACE), found exclusively in the testes, comprises a single catalytically active domain identical to the C-domain of sACE except for an N-terminal 67 amino acid residue gACE-specific sequence. Physiological functions of the two tissue-specific isozymes are not interchangeable (4). For example, sperm-specific expression of the gACE, not the sACE, in ACE knockout male mice restored fertility.

The human genome encodes ACE-2, also known as ACE homolog or ACEH, a structurally related but functionally diverse protein (5). ACE-2 is a type I membrane protein, but contains only a single active domain, with approximately 42% amino acid sequence identity to the N- and C-domains of sACE. ACE-2 has been shown to act as an essential regulator of heart function and a functional receptor for the SARS coronavirus, respectively (6, 7).

The Quantikine® Human ACE Immunoassay is a 4.5 hour solid phase ELISA designed to measure ACE in cell culture supernates, serum, plasma, and saliva. It contains NS0-expressed ectodomain of recombinant human sACE, and antibodies raised against the recombinant factor. Natural human ACE showed dose-response curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this kit can be used to determine relative levels of natural human ACE.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human ACE has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any ACE present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human ACE is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution 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 and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with the appropriate calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human ACE Microplate	892643	96 well polystyrene microplate (12 strips of 8 wells) coated with monoclonal antibody specific for human ACE.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human ACE Conjugate	892644	21 mL of a polyclonal antibody specific for human ACE conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human ACE Standard	892645	Recombinant human ACE in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1-34	895265	11 mL of a buffered protein base with preservative.	
Calibrator Diluent RD5-10	895266	21 mL of a buffered protein base with preservative. *For cell culture supernate/saliva samples.*	
Calibrator Diluent RD6-45	895850	21 mL of a buffered protein base with preservative. *For serum/plasma samples.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	Adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Collection device for saliva samples that has no protein binding or filtering capabilities such as a Salivette® or equivalent.
- Test tubes for dilution of standards and samples.
- Human ACE Controls (optional; R&D Systems®, Catalog # QC32).

PRECAUTIONS

ACE is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

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

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and 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 at room temperature 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 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.

Note: *EDTA and citrate plasma are not recommended for use in this assay due to their chelating properties.*

Saliva - Collect saliva using a collection device such as a Salivette or equivalent. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Saliva collector must not have any protein binding or filtering capabilities.*

SAMPLE PREPARATION

Serum and heparin plasma samples require a 10-fold dilution. A suggested 10-fold dilution is 20 µL of sample + 180 µL of Calibrator Diluent RD6-45.

Saliva samples require a 2-fold dilution. A suggested 2-fold dilution is 100 µL of sample + 100 µL of Calibrator Diluent RD5-10.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

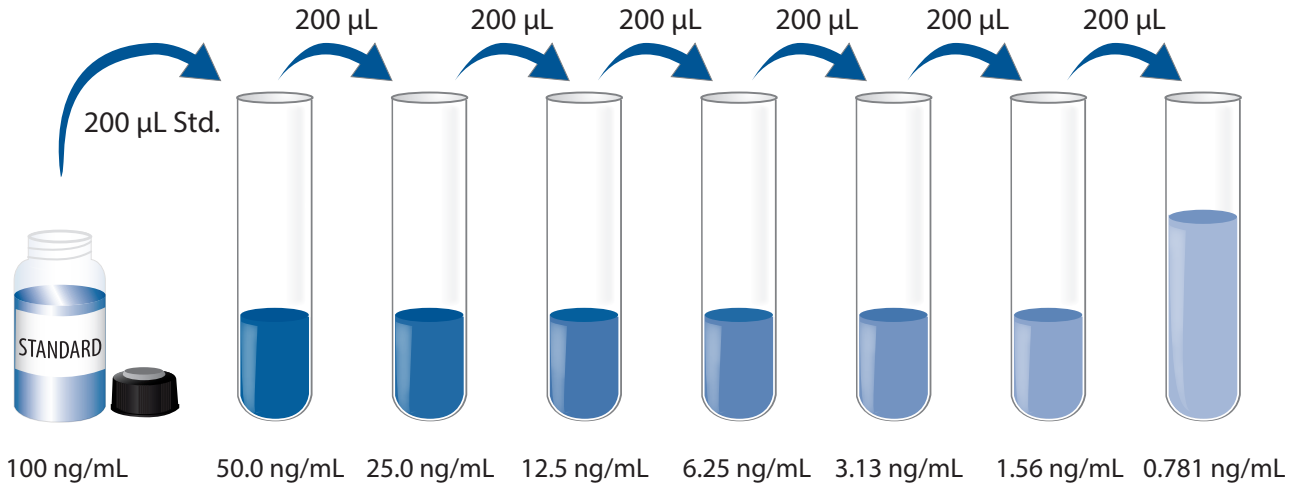
Note: *High concentrations of ACE are found in saliva. Use of a face mask and gloves to protect kit reagents from contamination is recommended.*

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Human ACE Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human ACE Standard with deionized or distilled water. This reconstitution produces a stock solution of 100 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 200 µL of Calibrator Diluent RD5-10 (*for cell culture supernate and saliva samples*) or Calibrator Diluent RD6-45 (*for serum/plasma samples*) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 50.0 ng/mL standard serves as the high standard. The appropriate calibrator diluent serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, samples, and controls be assayed in duplicate.

Note: *High concentrations of ACE are found in saliva. Use of a face mask and gloves to protect kit reagents from contamination is recommended.*

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1-34 to each well.
4. Add 50 µL of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 µL of Human ACE Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

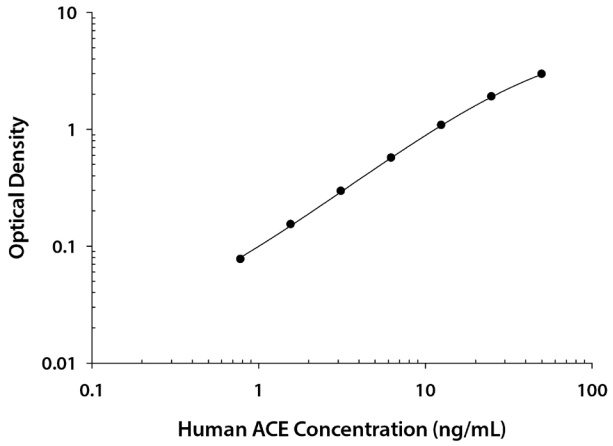
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human ACE concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

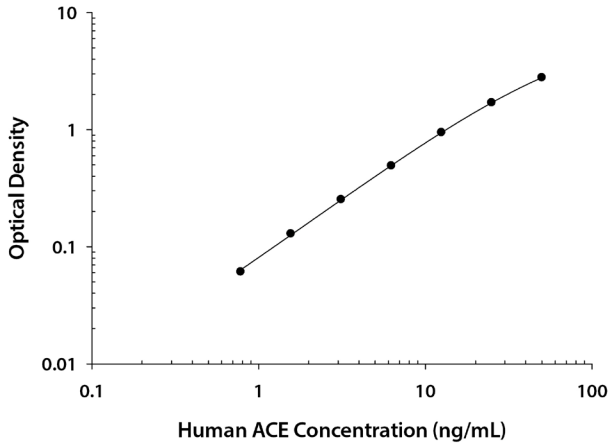
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE/SALIVA ASSAY



(ng/mL)	O.D.	Average	Corrected
0	0.015 0.015	0.015	—
0.781	0.090 0.093	0.092	0.077
1.56	0.164 0.171	0.168	0.153
3.13	0.309 0.310	0.310	0.295
6.25	0.578 0.588	0.583	0.568
12.5	1.091 1.098	1.095	1.080
25.0	1.902 1.918	1.910	1.895
50.0	2.955 2.988	2.972	2.957

SERUM/PLASMA ASSAY



(ng/mL)	O.D.	Average	Corrected
0	0.015 0.015	0.015	—
0.781	0.076 0.076	0.076	0.061
1.56	0.139 0.148	0.144	0.129
3.13	0.265 0.271	0.268	0.253
6.25	0.504 0.507	0.506	0.491
12.5	0.938 0.981	0.960	0.945
25.0	1.710 1.715	1.713	1.698
50.0	2.788 2.803	2.796	2.781

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

CELL CULTURE SUPERNATE/SALIVA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	40	40	40
Mean (ng/mL)	3.60	10.7	22.1	3.44	10.3	21.8
Standard deviation	0.08	0.30	0.95	0.21	0.40	0.87
CV (%)	2.2	2.8	4.3	6.1	3.9	4.0

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	40	40	40
Mean (ng/mL)	3.57	10.9	22.5	3.77	11.7	24.4
Standard deviation	0.12	0.43	0.90	0.29	0.60	1.20
CV (%)	3.4	3.9	4.0	7.7	5.1	4.9

RECOVERY

The recovery of human ACE spiked to three different levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=4)	98	89-106%
Serum* (n=4)	99	91-106%
Heparin plasma* (n=4)	100	94-106%

*Samples were diluted prior to assay as directed in the Sample Preparation section.

SENSITIVITY

Ninety assays were evaluated and the minimum detectable dose (MDD) of human ACE ranged from 0.008-0.051 ng/mL. The mean MDD was 0.019 ng/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

LINEARITY

To assess linearity of the assay, samples containing high concentrations of human ACE were diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay.

		Serum (n=4)	Heparin plasma* (n=4)	Saliva* (n=3)
1:2	Average % of Expected	106	101	109
	Range (%)	102-109	99-102	107-113
1:4	Average % of Expected	108	100	———
	Range (%)	103-112	96-105	———
1:8	Average % of Expected	109	102	———
	Range (%)	104-113	99-107	———
1:16	Average % of Expected	107	102	———
	Range (%)	105-109	95-108	———

*Samples were diluted prior to assay as directed in the Sample Preparation section.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human ACE produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma/Saliva - Samples from apparently healthy volunteers were evaluated for the presence of human ACE in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=41)	115.3	37.2-202	38.9
Heparin plasma (n=41)	123.4	58.1-211	37.6
Saliva (n=6)	2.26	0.91-4.70	1.30

Cell Culture Supernates:

Human peripheral blood cells (1 x 10^6 cells/mL) were cultured in DMEM supplemented with 5% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the cell culture supernates were removed on days 1 and 5 and assayed for natural human ACE. No detectable levels of human ACE were observed.

HUVEC human umbilical vein endothelial cells were grown to 90% confluency in EGM-2 media. Supernates were collected and concentrated 10-fold before testing. An aliquot of the cell culture supernate was removed, assayed for human ACE, and measured 2.35 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant human ACE.

The factors listed below were prepared at 500 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 500 ng/mL in a mid-range recombinant human ACE control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:	Recombinant mouse:
ACE-2	ACE-1
ECE-2	Kell
Neprilysin	Neprilysin

In addition to soluble ACE, this kit recognizes membrane-bound forms from lysates of cultured cells and supernates.

REFERENCES

1. Corvol, P. and T.A. Williams (1998) in *Handbook of Proteolytic Enzymes*, Barrett, A.J. *et al.* eds., Academic Press, San Diego, pp. 1066-1076.
2. Hu, J. *et al.* (2001) J. Biol. Chem. **276**:47863.
3. Turner, A.J. and N.M. Hooper (2002) Trends Pharmacol. Sci. **23**:177.
4. Kessler, S.P. *et al.* (2000) J. Biol. Chem. **275**:26259.
5. Guy, J.L. *et al.* (2003) Biochemistry **42**:13185.
6. Crackower, M.A. *et al.* (2002) Nature **417**:822.
7. Li, W. *et al.* (2003) Nature **426**:450.