

# Quantikine<sup>®</sup>

## 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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## 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  $\beta$ -peptide ( $A\beta$ ), retards  $A\beta$  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 recombinant Quantikine kit standards, indicating 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 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 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, dilute the samples with the appropriate Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Quantikine immunoassay, the possibility of interference cannot be excluded.

## MATERIALS PROVIDED

**ACE Microplate** (Part 892643) - 96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against ACE.

**ACE Conjugate** (Part 892644) - 21 mL of polyclonal antibody against ACE conjugated to horseradish peroxidase with preservatives.

**ACE Standard** (Part 892645) - 100 ng of recombinant human ACE in a buffered protein base with preservatives; lyophilized.

**Assay Diluent RD1-34** (Part 895265) - 11 mL of a buffered protein base with preservatives.

**Calibrator Diluent RD5-10** (Part 895266) - 21 mL of a buffered protein base with preservatives. *For cell culture supernate and saliva samples.*

**Calibrator Diluent RD6-45** (Part 895850) - 21 mL of a buffered protein base with preservatives. *For serum/plasma samples.*

**Wash Buffer Concentrate** (Part 895003) - 21 mL of a 25-fold concentrated solution of buffered surfactant with preservatives.

**Color Reagent A** (Part 895000) - 12.5 mL of stabilized hydrogen peroxide.

**Color Reagent B** (Part 895001) - 12.5 mL of stabilized chromogen (tetramethylbenzidine).

**Stop Solution** (Part 895032) - 6 mL of 2 N sulfuric acid.

**Plate Covers** - 4 adhesive strips.

## STORAGE

<b>Unopened Kit</b>	Store at 2 - 8° C. Do not use past kit expiration date.	
<b>Opened/ Reconstituted Reagents</b>	Diluted Wash Buffer	May be stored for up to 1 month at 2 - 8° C.*
	Stop Solution	
	Assay Diluent RD1-34	
	Calibrator Diluent RD5-10	
	Calibrator Diluent RD6-45	
	Conjugate	
	Unmixed Color Reagent A	
	Unmixed Color Reagent B	
	Standard	
	Microplate Wells	Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.*

\*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<sup>®</sup> or equivalent.
- Test tubes for dilution.
- Human ACE Controls (optional; available from R&D Systems).

## PRECAUTIONS

The Stop Solution provided with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

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

## SAMPLE COLLECTION AND STORAGE

**Cell Culture Supernates** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at  $\leq -20^{\circ}$  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  $\leq -20^{\circ}$  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  $\leq -20^{\circ}$  C. Avoid repeated freeze-thaw cycles.

**Note:** *EDTA and citrate plasma are not recommended anticoagulants 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  $\leq -20^{\circ}$  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  $\mu$ L sample + 180  $\mu$ L Calibrator Diluent RD6-45.

Saliva samples require a 2-fold dilution. A suggested 2-fold dilution is 100  $\mu$ L sample +100  $\mu$ L Calibrator Diluent RD5-10.

## REAGENT PREPARATION

Bring all reagents to room temperature before use.

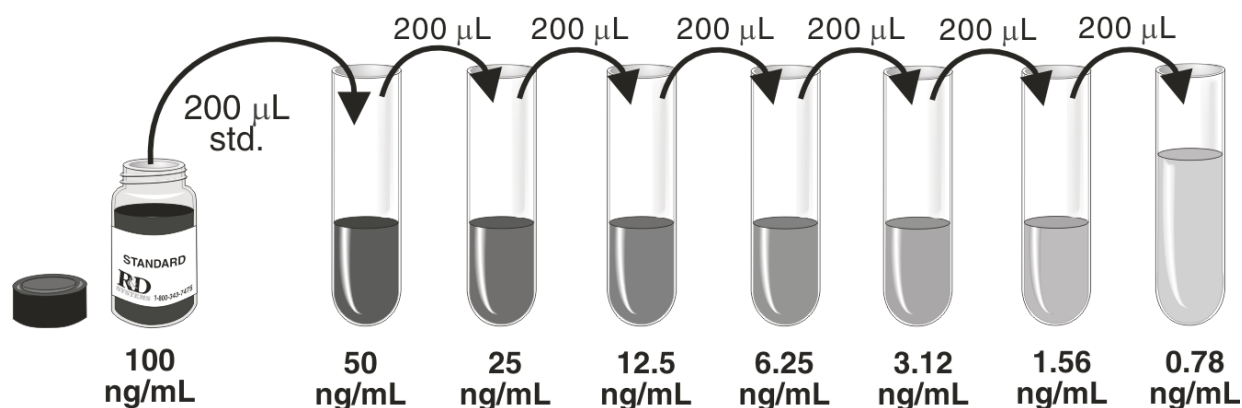
**Note:** High concentrations of ACE are found in saliva. Use of a face mask and gloves to protect kit reagents from contamination is recommended.

**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into 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  $\mu$ L of the resultant mixture is required per well.

**ACE Standard** - Reconstitute the ACE Standard with 1.0 mL of 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  $\mu$ L 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 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 samples, controls, and standards 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  $\mu\text{L}$  of Assay Diluent RD1-34 to each well.
4. Add 50  $\mu\text{L}$  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  $500 \pm 50$  rpm. A plate layout is provided to record standards and samples assayed.
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  $\mu\text{L}$ ) 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  $\mu\text{L}$  of 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  $\mu\text{L}$  of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 50  $\mu\text{L}$  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.

## ASSAY PROCEDURE SUMMARY

1. Prepare reagents, samples, and standards as instructed.



2. Add 100  $\mu\text{L}$  Assay Diluent RD1-34 to each well.



3. Add 50  $\mu\text{L}$  Standard, control, or sample\* to each well. Incubate 2 hours on the shaker at RT.



4. Aspirate and wash 4 times.



5. Add 200  $\mu\text{L}$  Conjugate to each well. Incubate 2 hours on the shaker at RT.



6. Aspirate and wash 4 times.



7. Add 200  $\mu\text{L}$  Substrate Solution to each well. Incubate 30 minutes **on the benchtop. Protect from light.**



8. Add 50  $\mu\text{L}$  Stop Solution to each well. Read at 450 nm within 30 minutes.  
 $\lambda$  correction 540 or 570 nm

\*Samples may require dilution. See Sample Preparation section

# CALCULATION OF RESULTS

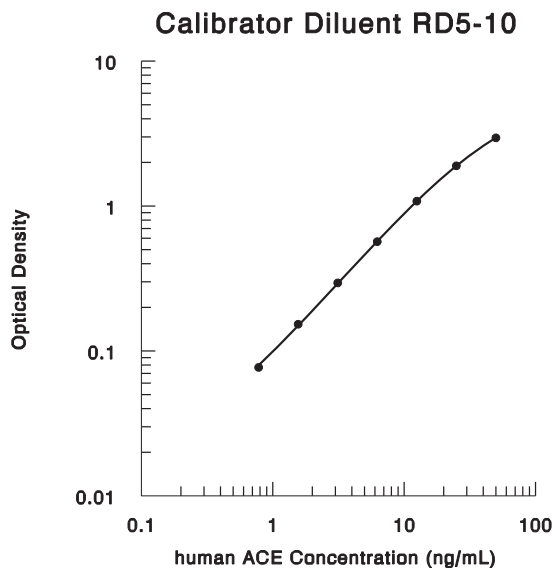
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

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 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.

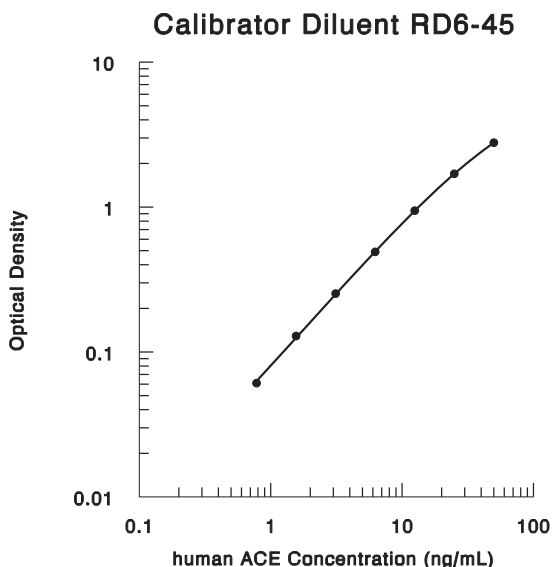
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

## TYPICAL DATA

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



ng/mL	O.D.	Average	Corrected
0	0.015 0.015 0.090	0.015	—
0.78	0.093 0.164	0.092	0.077
1.56	0.171 0.309	0.168	0.153
3.12	0.310 0.578	0.310	0.295
6.25	0.588 1.091	0.583	0.568
12.5	1.098 1.902	1.095	1.080
25	1.918 2.955	1.910	1.895
50	2.988	2.972	2.957



ng/mL	O.D.	Average	Corrected
0	0.015 0.015 0.076	0.015	—
0.78	0.076 0.139	0.076	0.061
1.56	0.148 0.265	0.144	0.129
3.12	0.271 0.504	0.268	0.253
6.25	0.507 0.938	0.506	0.491
12.5	0.981 1.710	0.960	0.945
25	1.715 2.788	1.713	1.698
50	2.803	2.796	2.781

## 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 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.

## 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.

### Serum/Plasma Assay

Sample	Intra-assay Precision			Inter-assay Precision		
	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	1.2
CV (%)	3.4	3.9	4.0	7.7	5.1	4.9

### Cell Culture Supernate/Saliva Assay

Sample	Intra-assay Precision			Inter-assay Precision		
	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

## RECOVERY

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

Sample	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 described in Sample Preparation.

## LINEARITY

To assess the linearity of the assay, samples containing high concentrations of ACE were serially 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 described in Sample Preparation.

## SENSITIVITY

Ninety assays were evaluated and the minimum detectable dose (MDD) of 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 optical density value of twenty zero standard replicates and calculating the corresponding concentration.

## 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 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 ( $1 \times 10^6$  cells/mL) were cultured in DMEM supplemented with 5% fetal calf serum, 50  $\mu$ M  $\beta$ -mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100  $\mu$ g/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10  $\mu$ g/mL PHA. Aliquots of the cell culture supernates were removed on days 1 and 5 and assayed for natural 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 natural ACE, and measured 2.35 ng/mL.

## SPECIFICITY

This assay recognizes recombinant and natural human ACE. The factors listed below were prepared at 500 ng/mL in Calibrator Diluents RD5-10 and RD6-45 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:**  
ACE-2  
ECE-2  
Nepriylsin

**Recombinant mouse:**  
ACE-1  
Kell  
Nepriylsin

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

## REFERENCES

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*Salivette is a registered trademark of Sarstedt, Inc.*

# PLATE LAYOUT

Use this plate layout as a record of standards and samples assayed.

1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
	<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>	<b>F</b>	<b>G</b>	<b>H</b>

# NOTES