APPLICATION GUIDE

Human ACE2 ELISA Kit

Catalog No. EA200003

For quantitative detection of human ACE2 in cell culture supernates, serum and heparin-plasma

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURE

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Principle of the Assay

Angiotensin I-converting enzyme 2 (ACE2) is a protein that belongs to the angiotensin-converting enzyme family of dipeptidyl carboxydipeptidases and has considerable homology to human angiotensin 1 converting enzyme. This secreted protein catalyzes the cleavage of angiotensin I into angiotensin 1-9 and angiotensin II into the vasodilator angiotensin 1-7. The organ- and cell-specific expression of this gene suggests that it may play a role in the regulation of cardiovascular and renal function, as well as fertility. In addition, the encoded protein is a functional receptor for the spike glycoprotein of the human coronaviruses HCoV-NL63 and the human severe acute respiratory syndrome coronaviruses, SARS-CoV and SARS-CoV-2.

This sandwich ELISA is used to measure human ACE2 in cell culture supernatant, serum and heparin-plasma samples. Microtitration wells coated with anti-human ACE2 capture antibody are exposed to test specimens. The ACE2 antigen in the specimen is specifically captured onto the immobilized antibody during specimen incubation. The captured ACE2 antigen is then reacted with a biotinylated human ACE2 detection antibody. Subsequently, Streptavidin-HRP conjugate is then added. After wash, specifically bound enzyme conjugate is detected by reaction with the Substrate Solution, tetramethylbenzidine (TMB). The assay is measured spectrophotometrically to indicate the level of ACE2 present in a sample.

Kit Presentation

Materials Supplied

The reagents supplied in this pack are for Research Use Only.

Description	Quantity
ACE2 Antibody Coated 96-well Plate in foil pouch with desiccant	1
Recombinant human ACE2 Standard (250ng/mL)	0.1 mL
Biotinylated ACE2 Detection Antibody (100x)	120 µL
Streptavidin Conjugated Horseradish Peroxidase (100x)	120 µL
Assay Buffer	30 mL
Sample Diluent	12 mL
Substrate Solution (TMB)	12 mL
Stop Solution (1N HCl)	12 mL
Wash Buffer (20x)	60 mL
Plate Sealer	3

Additional Requirements for Manual Processing

1. Horizontal orbital plate shaker capable of maintaining a speed of 450±50 rpm.
2. Disposable tip micropipettes to deliver volumes of 5µL, 10 µL, 25 µL, 100 µL and 200 µL (multichannel pipette preferred for dispensing reagents into microtiter plates).

3. Distilled or deionized water.
4. Clean, disposable plastic/glass test tubes, approximate capacities 5mL and 10mL.
5. Range of standard, clean volumetric laboratory glassware consisting of, at least, 15 mL and 100 mL beakers, 1 L graduated cylinder, 1 mL, 5 mL, and 10 mL pipettes.
6. Absorbent paper towels.
7. Automatic microplate washer or laboratory wash bottle.
8. Microplate reader with 450nm filter.
9. Latex gloves, safety glasses and other appropriate protective garments.
10. Biohazard waste containers.
11. Safety pipetting devices for 1 mL or larger pipettes.
12. Timer.

Storage and Stability

All reagents should be stored at 2-8°C and should not be used beyond the expiration date on the label. Once opened, microtitration strips may be stored at 2-8°C until the expiration date on the label, provided that desiccated conditions are maintained. Unused strips should be returned to their original foil pouch along with the sachet of desiccant. Secure open foil pouch using zip top before storage. The working strength Wash Buffer should not be stored for longer than 3 weeks at 2-8°C. It is recommended that Wash buffer be freshly diluted before each assay. If the working strength buffer becomes visibly cloudy or develops precipitate during the 3 weeks, do not use it.

Indications of Deterioration

The human ACE2 Assay kit may be considered to have deteriorated if:

1. The kit fails to meet the required criteria for a valid test (see Interpretation of Results).
2. Reagents becoming visibly cloudy or develop precipitate. Note: Concentrated Wash buffer, when cold, normally develops crystalline precipitates, which re-dissolve on heating at 37°C.
3. The Substrate Solution turns dark blue. This is likely to be caused by chemical contamination of the Substrate Solution.

Warnings and Precaution

Safety

1. The reagents supplied in this kit are for ***Research use only***.

2. Caution: All blood products should be treated as potentially infectious.

Essential precautions can be summarized as follows:

>do not pipette by mouth.

>Wear disposable gloves during all specimen and assay manipulations.

>Avoid use of sharp or pointed liquid handling devices, which may puncture skin.

>Do not smoke, eat or drink in the laboratory work area.

>Avoid splashing of liquid specimens and reagents and the formation of aerosols.

>Wash hands thoroughly on completion of a manipulation.

>The Centers for Disease Control & Prevention and the National Institutes of Health recommend that potentially infectious agents be handled at Biosafety Level 2.

3. The kit contains reagent systems which are optimized and balanced for each kit lot. Do not interchange reagents from kits with different lot numbers. Do not interchange vial caps or stoppers either within or between kits.

4. The Substrate Solution and Stop Solution in this kit contain ingredients that can irritate the skin and cause eye damage. Handle them with care and wear suitable protective clothing and eye/face protection. In case of contact with skin or eyes, immediately flush the affected area with plenty of water. For eyes, obtain medical attention.

5. Disposal or decontamination of fluid in the waste reservoir from either the plate washer or trap for vacuum line in the manual system should be in accordance with guidelines set forth in the Department of Labor, Occupational Safety and Health Administration, occupational exposure to blood-borne pathogens; final rule (29 CFR 1910,1030) FEDERAL REGISTER, pp. 64176-84177,12/6/91.

Technical Suggestions

1. This kit should be used in strict accordance with the instructions in the Package Insert.
2. Do not use the kit after the expiration date printed on the outer carton label.
3. Do not cross contaminate reagents.
4. To ensure accurate results and avoid cross-contamination, use proper adhesive plate sealers during incubation steps, and change pipette tips when adding each standard and sample. Multi-channel pipettes are recommended for large assays. Always use fresh pipette tips when drawing from stock reagent bottles.
5. All reagents should be added to the plate in the same order.
6. Protect Substrate Solution from light.
7. If the Stop Solution does not mix thoroughly with the Substrate Solution, the color in the wells may appear green after adding stop solution. Gently tap the plate or pipette up and down to mix until the color in the wells change to yellow (avoid bubbles during this step).
8. Always use clean, preferably disposable, glassware for all reagent preparation.
9. Allow foil bags to warm to room temperature before opening. This avoids condensation on the inner surface of the bag, which may contribute to a deterioration of coated strips intended for future use.
10. Reagents should be dispensed with the tip of the micropipettes touching the side of the well at a point about mid-section. Follow manufacturer's recommendations for automatic processors.
11. Always keep the upper surface of the microtitration strips free from excess fluid droplets. Reagents and buffer over-spill should be blotted dry on completion of the manipulation.
12. Do not allow the wells to completely dry during an assay.

13. Automatic or semi-automatic EIA processors or liquid handling systems should be qualified specifically for use with the assay by demonstration of equivalence to the manual processing methods.
14. Consistent with good laboratory practice, it is recommended that all pipetting devices (manual or automatic), timers and thermometers are regularly calibrated according to the manufacturer's instructions.
15. Care must be taken to ensure that specimens are dispensed correctly to each test well. If a specimen is inadvertently not added to a well, the result for that well will be non-reactive, regardless of the actual result of the specimen.

Method of Use

Specimen Collection and Storage

The Human ACE2 ELISA is intended for use with cell culture supernatants, serum and heparin-plasma. The specimen should be tested as soon as possible. However, if the specimen needs storage, the specimens should be stored frozen at -20°C or below. Do not use self-defrosting freezers. Specimens that have been frozen and thawed should be thoroughly mixed before testing.

Cell Culture Supernatant - 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.

Rinse Cycle

Efficient rinsing to remove un-complexed components is a fundamental requirement of enzyme immunoassay procedures. Automatic plate washers may be used provided they meet the following criteria: 1. All wells are completely aspirated. 2. All wells are filled to the rim (300 μL) during the rinse cycle. 3. Wash buffer is dispensed at a good flow rate. 4. The microtitration plate washer must be well maintained to prevent contamination from previous use. Manufacturer’s cleaning procedures must be followed diligently

For the rinse cycle, the machine should be set to four consecutive washes. On completion of the cycle, invert the microtitration plate and tap firmly on absorbent paper towels. Check for any residual Wash buffer in the wells and blot dry the upper surface of the wells with a paper towel.

Alternatively, the following manual system may be employed:

1. Discard or aspirate well contents using a vacuum line fitted with a trap.

2. Fill all wells to the brim with wash buffer dispensed from a squeeze-type laboratory wash bottle.

3. Discard or aspirate fluids.

4. Repeat steps 2 and 3, three times.

5. Invert the microtitration plate and tap firmly on absorbent paper towels.

Preparation for the Assay

1. *Standard preparation*

Prepare protein standard by diluting 10µL of standard stock into 490 µL (1:50 dilution) of assay buffer. This will give a final concentration of 5000 pg/mL as shown in Table 1

2. *Sample preparation*: neat serum or plasma sample is recommended for the assay. Proper dilution using sample diluent is needed for samples with ACE2 concentration beyond the highest detection range.

3. *Detection antibody preparation:* dilute the concentrated biotin conjugated detection antibody 1:100 using assay buffer.

4. *SA-HRP preparation*: dilute the concentrated streptavidin HRP conjugate 1:100 using assay buffer.

5. *Wash buffer*

Prepare working-strength Wash buffer by diluting 1 part concentrate with 19 parts of distilled or de-ionized water. If a kit is likely to be utilized over a period in excess of 4 weeks, then it is recommended that only enough stock concentrate be diluted sufficient for immediate needs.

Quantitative Assay Procedure

To test quantitatively, a standard curve should be prepared using assay diluent as shown in the table below. Each standard should be run in duplicate.

Table 1: Human ACE2 Quantitative Standard Curve Generation

Standard Number	Concentration of ACE2 (pg/mL)	ACE2 Standard (µL)	Assay Diluent (µL)
1	5000	10	490
2	2500	250 of #1	250
3	1250	250 of #2	250
4	625	250 of #3	250
5	312.5	250 of #4	250
6	156.25	250 of #5	250
7	78.13	250 of #6	250
8	0		250

Assay Procedure

1. Allow all reagents to reach room temperature (18-25°C).

2. Select sufficient microtitration well strips to accommodate all test specimens, controls and reagent blank. Fit the strips into the holding frame. Label wells according to specimen identity using the letter/number cross-reference system molded into the plastic frame.

3. Dispense 100 µL of each standard and sample into appropriate wells. Note: All standards and samples should be tested in duplicate. Note: Depending on the ACE2 concentration of your sample, dilution using sample diluent may be needed. If the sample ACE2 concentration is not known, you can titrate the original sample.

4. Incubate for 2 hours at room temperature with moderate shaking (450±50rpm) on a horizontal orbital plate shaker.

5. Wash the microtitration plate 4 times as described in the Rinse Cycle section.

6. Pipette 100 µL of working concentration detection antibody into each well and incubate for 1 hour at room temperature with moderate shaking (450±50rpm) on a horizontal orbital plate shaker.

7. Wash the microtitration plate 4 times as described in the Rinse Cycle section.

8. Pipette 100 µL of working concentration Streptavidin HRP conjugate into each well and incubate for 30 min at room temperature with moderate shaking (450±50rpm) on a horizontal orbital plate shaker.

11. Wash the microtitration plate 4 times as described in the Rinse Cycle section.

12. Dispense 100 µL Substrate Solution into each well. A multichannel pipette should be used for best results. Leave at room temperature (18-25°C) and protected from direct sunlight for 25 minutes.

13. Stop the reaction by adding 100 µL of Stop Solution to each well. The blue solution should change to a uniform yellow color. Ensure that the undersides of the wells are dry and that there are no air bubbles in the well contents.

14. Immediately after adding the Stop solution, read the absorbance values at 450 nm using a microtitration plate reader.

Interpretation of Results

Quantitative Analysis

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

A 4-parameter logistic (4-PL) or a linear regression model providing a point to point curve fitting provides acceptable results. Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic (4-PL) or a

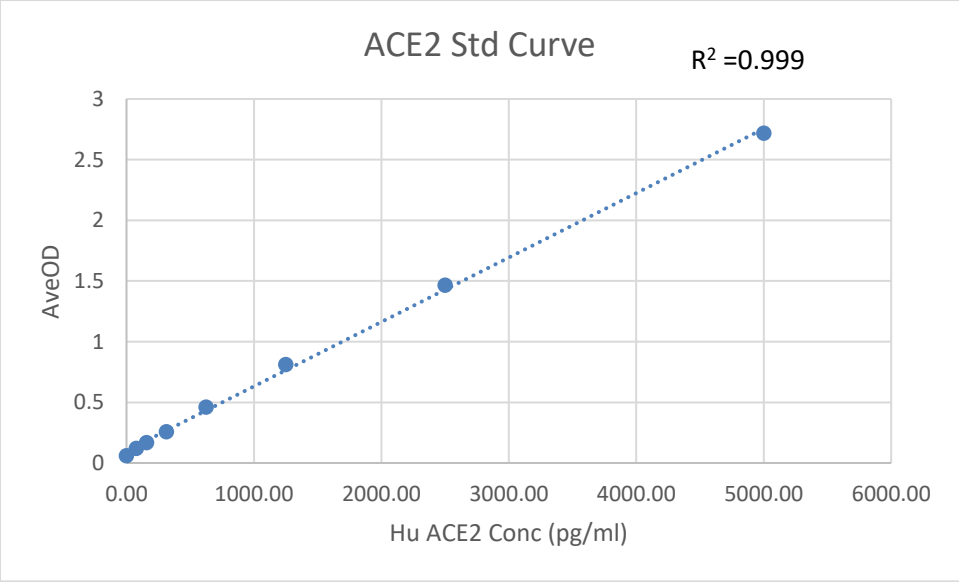
linear regression curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. Do not force the line to be linear. The concentration of the specimens can be found directly from the standard curve.

Table 2. Example Data at 450nm.

Standards	450 nm absorbance
Standard 1 (0 pg/mL)	0.06
Standard 2 (78.13 pg/mL)	0.12
Standard 3 (156.25 pg/mL)	0.17
Standard 4 (312.5 pg/mL)	0.26
Standard 5 (625 pg/mL)	0.46
Standard 6 (1250 pg/mL)	0.81
Standard 7 (2500 pg/mL)	1.47
Standard 8 (5000 pg/mL)	2.72

Typical Human ACE2 ELISA Kit Standard Curve

This standard curve was generated at OriGene for demonstration purpose only. A standard curve must be run with each assay.



Note: This standard curve is only an example and should not be used to generate any results.

Performance Characteristics

1. **Recovery**
   The recovery of human ACE2 spiked to three different-levels of the assay range in diluted samples was evaluated

Sample Type	Average % Recovery
Serum	95
Heparin-plasma	91
Cell culture media	95

2. **Linearity**
   To assess the linearity of the assay, neat or human ACE2 spiked samples were diluted to produce samples with values within the dynamic range of the assay.

		Cell culture media	Heparin plasma	Serum
1:2	%Expected	106	109	96
1:4	%Expected	103	104	89
1:8	%Expected	94	98	112

3. **Sensitivity:** 19.88pg/mL

4. **Precision**

   Three serum and plasma samples with different levels of ACE2 were assayed 10 times each on three different assays. The intra-assay CV percentage and inter-assay CV percentage were calculated.

Sample	%CV in Assay 1	%CV in Assay 2	%CV in Assay 3	Ave %CV
Serum 1 (n=10)	3.55	2.53	2.28	2.79
Serum 2 (n=10)	5.79	3.27	4.26	4.44
Serum 3 (n=10)	8.91	4.25	5.83	6.33
Heparin-Plasma 1 (n=10)	5.66	4.68	7.00	5.78
Heparin-Plasma 2 (n=10)	7.12	3.24	6.25	5.54
Heparin-Plasma 3 (n=10)	6.72	9.12	13.24	9.70

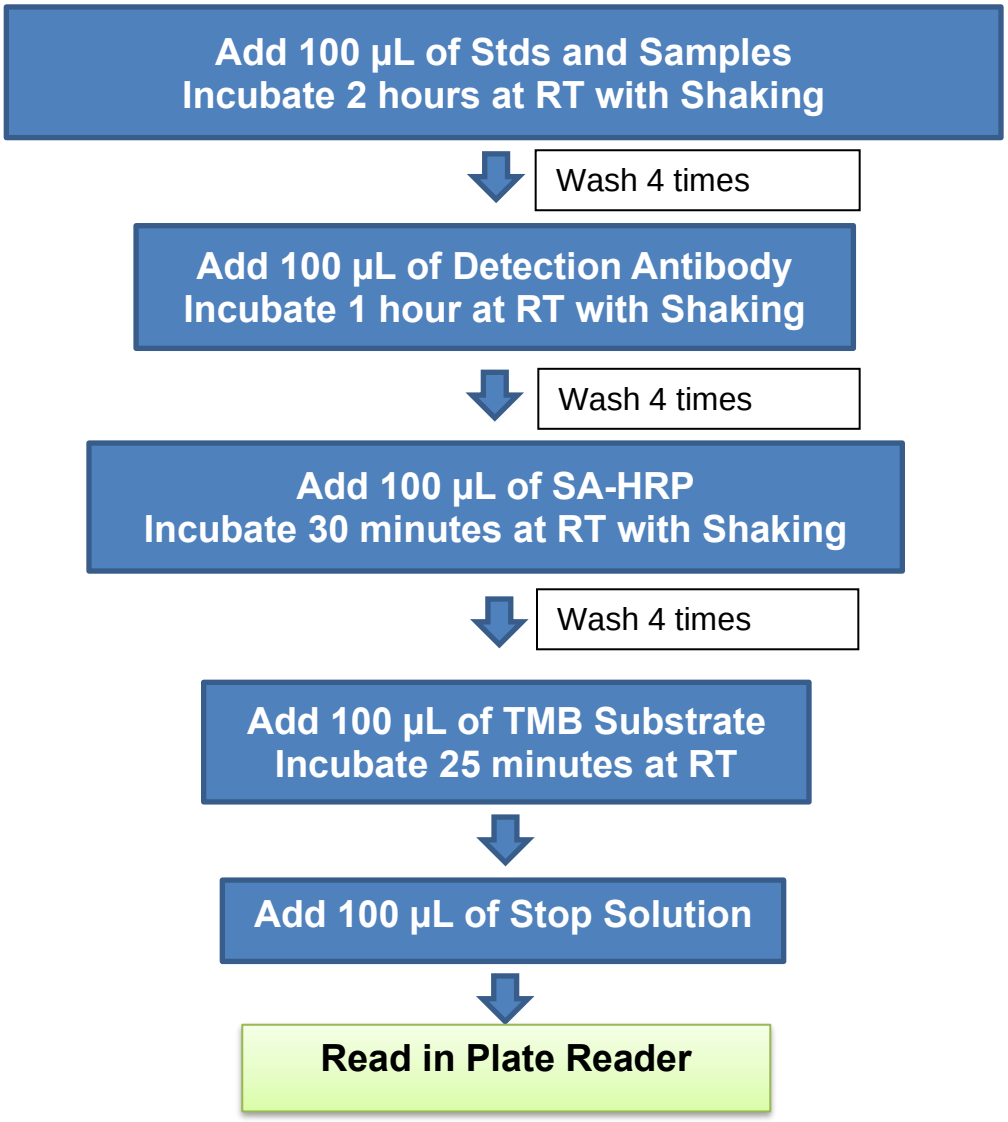
Sample	Mean (pg/ml) in assay1	Mean (pg/ml) in assay2	Mean (pg/ml) in assay3	Ave (pg/ml)	SD	%CV
Serum 1 (n=10)	826.88	788.68	871.12	828.89	41.26	4.98
Serum 2 (n=10)	483.00	443.24	457.66	461.30	20.13	4.36
Serum 3 (n=10)	305.51	297.71	297.57	300.26	4.55	1.51
Heparin-Plasma 1 (n=10)	188.53	200.69	196.19	195.14	6.15	3.15
Heparin-Plasma 2 (n=10)	152.14	162.02	167.83	160.66	7.93	4.94
Heparin-Plasma 3 (n=10)	118.27	125.25	124.31	122.61	3.79	3.09

Limitations of Use

1. Assay values determined using assays from different manufacturers or different methods may not be used interchangeably.

3. The assay cannot be used to quantitate samples with ACE2 assay values greater than the highest standard without further serial dilution of the samples. See the Interpretation of Results section for directions on testing such samples.

Assay Flowchart



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