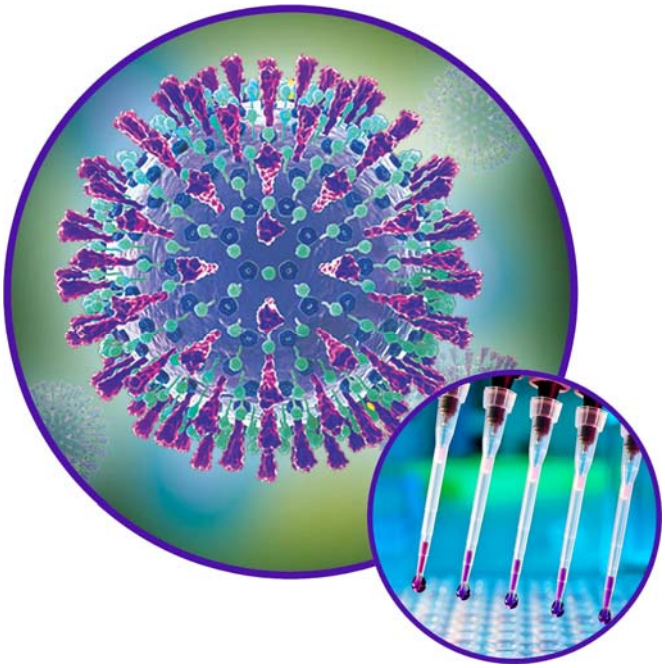


abeomics
Antibodies & Engineered Cell Lines™

MANUAL

ACE2 (human) ELISA Kit

[Angiotensin-Converting Enzyme 2 (human) ELISA Kit]

For research use only. Not for diagnostic use

Cat.No.90-7008

Table of Contents

1. Intended Use 3

2. Introduction 3

3. General References 4

4. Assay Principle 5

5. Handling & Storage 5

6. Kit Components 5

7. Materials Required but *Not* Supplied 6

8. General ELISA Protocol 7

   Preparation and Storage of Reagents 7

   Sample collection, storage and dilution 8

   Assay Procedure (Checklist) 9

9. Calculation of Results 10

10. Typical Data 10

11. Performance Characteristics 11-12

12. Technical Hints and Limitations 13

13. Troubleshooting 14

14. Notes 15

1. Intended Use

The ACE2 (human) ELISA Kit is to be used for the *in-vitro* quantitative determination of human ACE2 in cell culture supernatants, serum, plasma and urine. This ELISA Kit is for research use only.

2. Introduction

Human ACE2 gene, localized in X chromosome, encodes for a 805 amino acid protein with an N-terminal signal sequence, a metalloprotease zinc binding site (HEMGH) and a hydrophobic region near the C-terminus acting as a membrane anchor (transmembrane domain). Angiotensin-converting enzyme 2 (ACE2) is a type I transmembrane metallocarboxypeptidase within the renin-angiotensin system (RAS), which plays a key role in blood pressure regulation, fluid and electrolyte balance, thirst, cardiac/renal function and growth (1). ACE2 is expressed on the cell surface of type 2 alveolar epithelial cells in the lungs as well as on cells in many other tissues (2). ACE2 shares approximately 60% homology with ACE, the other key enzyme of the RAS system (3).

ACE2 converts angiotensin II (Ang II) into Ang (1-7), which acts on the Mas receptor and plays a role in cardiovascular disease to lower blood pressure through vasodilation and by promoting kidney sodium and water excretion, but also to lower inflammation (4). The effects of ACE2 directly oppose those induced by ACE-Ang II signaling, whereby ACE converts Ang I into Ang II, which increases blood pressure by inducing vasoconstriction, increasing kidney reabsorption of sodium and water and promoting inflammation.

ACE2 has been identified as a key receptor on target cells for SARS-CoV infections in 2002 (5). ACE2 functions as the entry receptor of the new SARS-CoV-2 coronavirus that emerged in China in 2019 and is the cause of the new disease COVID-19. Strong binding of the spike protein of SARS- CoV-2 to ACE2, along with proteolytic cleavage of ACE2 by transmembrane serine protease 2 (TMPRSS2), facilitates entry of the virus into cells, viral replication and cell-to-cell transmission.

ACE2 can undergo an ADAM17 (*a disintegrin and metalloproteinase 17*)-mediated“ shedding” from endothelial cells, resulting in the release of the ectodomain into the circulation (6).This soluble form may act as a competitive interceptor of SARS-CoV-2 and other coronaviruses by preventing binding of the viral particle to the surface-bound, full-length ACE2 (7). Soluble ACE2 might also be used as biomarker of hypertension and cardiovascular diseases (6).

3. General References

(1) Intrarenal renin-angiotens in system in regulation of glomerular function:L.G.Navar; Curr.Opin. Nephrol. Hypertens. **23,** 38 (2014)

(2) Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis: I. Hamming, et al.; J. Pathol. **203**, 63 (2004)

(3) A human homolog of angiotensin-converting enzyme: cloning and functional expression as a captopril-insensitive carboxypeptidase: S.R. Tipnis, et al.; J. Biol. Chem. **275**, 33238 (2000)

(4) Angiotensin-converting enzyme2-a new cardiac regulator: M.Boehm,etal.; NewEng.J.Med. **347**,1795(2002)

(5) SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor: M. Hoffmann, et al.; Cell **181**, 271 (2020)

(6) Circulating ACE2 in Cardiovascular and Kidney Diseases: L.Anguiano,etal., Curr.Med.Chem. **24**, 3231(2017)

(7) Inhibition of SARS-CoV-2 Infections in Engineered Human Tissues Using Clinical-Grade Soluble Human ACE2: V. Monteil, et al., Cell **181**, 905 (2020)

4. Assay Principle

This assay is a sandwich Enzyme Linked-Immunosorbent Assay (ELISA) for quantitative determination of human ACE2 in cell culture supernatants, serum, plasma and urine. A polyclonal antibody specific for human ACE2 has been pre-coated on to the 96-well microtiter plate. Standards (STD) and samples are pipette into the wells for binding to the coated antibody. After extensive washing to remove unbound compounds, ACE2 (h) is recognized by the addition of a biotinylated monoclonal antibody specific for human ACE2 (DET). After removal of excess biotinylated antibody, streptavidin-peroxidase (STREP-HRP) is added. Following a final washing, peroxidase activity is quantified using the substrate 3,3',5,5'- tetramethylbenzidine (TMB). The intensity of the color reaction is measured at 450nm after acidification and is directly proportional to the concentration of ACE2 in the samples.

5. Handling & Storage

- Reagent must be stored at 2-8°C when not in use
- Plate and reagents should be at room temperature before use.
- Do not expose reagents to temperatures greater than 25°C.

6. Kit Components

• 1 vial human ACE2 Standard (lyophilized)	(100 ng)	(STD)
• 1 vial ACE2 Detection Antibody	(20 µl)	(DET)
• 1 vial HRP Labeled Streptavidin (lyophilized)	(2 µg)	(STREP-HRP)
• 2 bottles Wash Buffer 10X	(2x30ml)	(Wash Buffer 10X)
• 1 bottle Sample Buffer 5X	(1x30ml)	(Sample Buffer 5X)
• 1 bottle TMB Substrate Solution	(12ml)	(TMB)
• 1 bottle Stop Solution	(12ml)	(STOP)
• 1 plate coated with ACE2 Antibody	(6x16-wellstrips)	
• 2 plate Covers (plastic film)		
• 2 silica Gel Mini bags		

7. Materials Required but *Not* Supplied

- Microtiter plate reader at 450nm
- Calibrated precision pipettes. Disposable pipette tips
- Deionized water
- Microtubes or equivalent for preparing dilutions
- Disposable plastic containers for preparing working buffers
- Plate washer: automated or manual
- Glass or plastic tubes for diluting and aliquoting standard

8. General ELISA Protocol

Preparation and Storage of Reagents

NOTE: *Prepare just the appropriate amount of the buffers necessary for the assay.*

- **Wash Buffer 10X** has to be diluted with deionized water 1:10 before use (e.g. 30 ml Wash Buffer 10X + 270 ml water) to obtain Wash Buffer 1X.
- **Sample Buffer 5X** has to be diluted with deionized water 1:5 before use(e.g.20ml Sample Buffer 5X + 80 ml water) to obtain Sample Buffer 1X.
- **Detection Antibody (DET)** has to be diluted to 1:1000 in Sample Buffer 1X (10 µl DET +10 ml Sample Buffer 1X).

  NOTE: *The diluted Detection Antibody is not stable and cannot be stored!*

- **HRP Labeled Streptavidin (STREP-HRP)** has to be reconstituted with 100 µl of Sample Buffer 1X.
  - After reconstitution of STREP-HRP, prepare aliquots and store the mat-20°C. **Avoid freeze/thaw cycles**.
  - Dilute the reconstituted STREP-HRP to the working concentration by adding 50µl in 10 ml of Sample Buffer 1X (1:200).

  NOTE: *The diluted STREP-HRP is not stable and cannot be stored!*

- **Human ACE2 Standard (STD)** has to be reconstituted with100µl of Sample Buffer 1X.
  - This reconstitution produces a stock solution of 1 µg/ml. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes **at room temperature.** Mix well prior to making dilutions.

  NOTE: *There constituted standard is aliquoted and stored at-20°C!*

  - Dilute the standard protein concentrate (STD) (**1 µg/ml**) in Sample Buffer 1X. A seven-point standard curve using 2-fold serial dilutions in Sample Buffer 1X is recommended.

  - Suggested standard points are:
    4, 2, 1, 0.5., 0.25, 0.125, 0.0625, and 0ng/ml.

Start with the dilution of the concentrate (STD):

To obtain	Add	Into
10ng/ml	10µl of ACE2(STD) (1µg/ml)	990µl of Sample Buffer 1X

Dilute further for the standard curve:

To obtain	Add	Into
4ng/ml	240µl of ACE2 (10ng/ml)	360µlofSampleBuffer1X
2ng/ml	300µl of ACE2 (4ng/ml)	300µlofSampleBuffer 1X
1ng/ml	300µl of ACE2 (2ng/ml)	300µlofSampleBuffer 1X
0.5ng/ml	300µl of ACE2 (1ng/ml)	300µlofSampleBuffer 1X
0.25ng/ml	300µl of ACE2 (0.5ng/ml)	300µlofSampleBuffer 1X
0.125ng/ml	300µl of ACE2 (0.25ng/ml)	300µl of Sample Buffer 1X
0.0625ng/ml	300µl of ACE2 (0.125ng/ml)	300µl of Sample Buffer 1X
0ng/ml	300µl of Sample Buffer 1X	Empty tube

Sample collection, storage and dilution

Serum: Use a serum separator tube. Let samples clot at room temperature for 30 minutes before centrifugation for 20minutes at 1,000xg. Assay freshly prepared serum or store serum in aliquot at ≤-20°C for later use. Avoid repeated freeze/thaw cycles.

Plasma: Collect plasma using heparin, citrate or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay freshly prepared plasma or store plasma sample in aliquot at ≤ -80°C for later use. Avoid repeated freeze/ thaw cycles.

Urine: Aseptically collect the urine of the day, void directly into a sterile container. Assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze/thaw cycles.

Serum, Plasma, Urine and Cell Culture Supernatant have to be diluted in Sample Buffer1X. Samples containing visible precipitates must be clarified before use.

NOTE: *As a starting point, ½ dilution of serum or of plasma is recommended! For Urine,1/4 dilution is recommended. If sample values fall outside the detection range of the assay, a lower or higher dilution may be required!*

Assay Procedure (Checklist)

☐	1. Determine the number of 16-well strips needed for the assay and insert them in the frame for current use. The extra strips are left in the bag with 2 silica gel mini bags and stored at 4°C. **NOTE:** *Remaining16-well strips coated with ACE2 antibody when opened can be stored in the presence of 2 silica gel mini bags at 4°C for up to 1 month.*
☐	2. Add 100 µl of the different standards into the appropriate wells in duplicate! At the same time, add 100 µl of diluted plasma, serum, urine or cell culture supernatant samples in duplicate to the wells **(see8.1.Preparation and Storage of Reagents and 8.2 Preparation of Samples).**
☐	3. Cover the plate with plastic film and incubate for **2 hours at Room Temperature.**
☐	4. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or auto-washer. Repeat the process for a total of five washes. After the last wash, complete removal of liquid is essential for good performance.
☐	5. Add100µl to each well of the diluted Detection Antibody **(DET) (see8.1 Preparation and Storage of Reagents)**.
☐	6. Cover the plate with plastic film and incubate for **1hour at Room Temperature.**
☐	7. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or auto-washer. Repeat the process for a total of five washes. After the last wash, complete removal of liquid is essential for good performance.
☐	8. Add100µl to each well of the diluted HRP Labeled Streptavidin **(STREP-HRP) (see 8.1. Preparation and Storage of Reagents)**.
☐	9. Cover the plate with plastic film and incubate for **30 minutes at Room Temperature**.
☐	10. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or auto-washer. Repeat the process for a total of five washes. After the last wash, complete removal of liquid is essential for good performance.
☐	11. Add 100µl to each well of TMB substrate solution **(TMB)**.
☐	12. Allow the color reaction to develop **at Room Temperature in the dark for 20-25 minutes**. Do not cover the plate.
☐	13. Stop the reaction by adding 100µl of Stop Solution (**STOP**).Tap the plate gently to ensure thorough mixing. The substrate reaction yields a blue solution that turns yellow when Stop Solution (**STOP**) is added.
	! CAUTION: CORROSIVE SOLUTION!
☐	14. Measure the OD at 450 nm in an ELISA reader.

9. Calculation of Results

- Average the duplicate readings for each standard and sample and subtract the average blank value (obtained with the 0 ng/ml point).
- Generate the standard curve by plotting the average absorbance obtained for each standard concentration on the horizontal (X) axis vs. the corresponding ACE2 concentration (ng/ml) on the vertical axis (see **10.** TYPICAL DATA).
- Calculate the ACE2 concentrations of samples by interpolation of the regression curve formula as shown above in a form of a quadratic equation
- If the test sample was diluted, multiply the interpolated value by the dilution factor to calculate the concentration of human ACE2 in the sample.

10. Typical Data

The following data are obtained using the different concentrations of standard as described in this protocol:

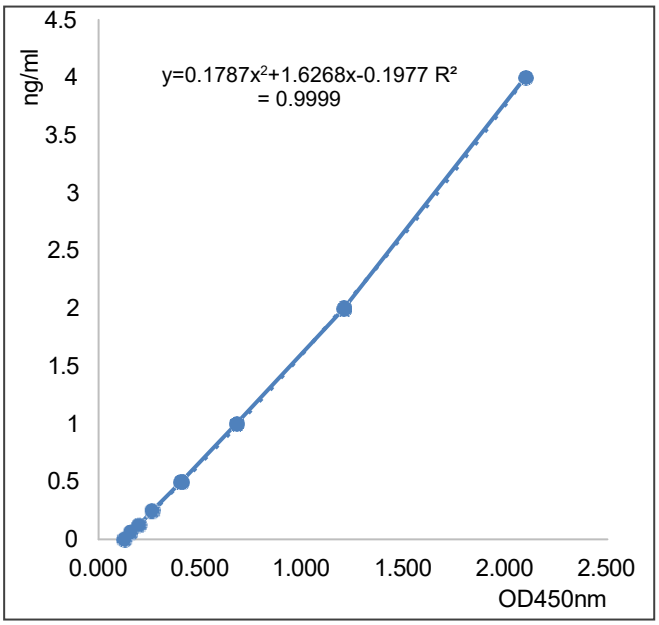


Figure: Standard curve

Standard ACE2 (ng/ml)	Optical Density (mean)
4	2.095
2	1.204
1	0.676
0.5	0.405
0.25	0.263
0.125	0.198
0.0625	0.158
0	0.127

11. Performance Characteristics

A. Sensitivity (Limit of detection):

The lowest level of human ACE2 that can be detected by this assay is **40pg/ml**.

NOTE: *The Limit of detection was measured by adding three standard deviations to the mean value of 50 zero standard.*

B. Assay range: 0.0625ng/ml–4ng/ml

C. Specificity:

This ELISA is specific for the measurement of natural and recombinant human ACE2.

D. Intra-assay precision:

Four samples of known concentrations of human ACE2 were assayed in replicates 6 times to test precision within an assay.

Samples	Means (ng/ml)	SD	CV(%)	n
A1	2.02	0.04	2.04	6
A2	0.98	0.04	3.69	6
A3	2.00	0.037	1.88	6
A4	1.45	0.075	5.22	6

E. Inter-assay precision:

Four samples of known concentrations of human ACE2 were assayed in 4 separate assays to test precision between assays.

Samples	Means(ng/ml)	SD	CV(%)	n
B1	0.50	0.01	2.35	4
B2	0.276	0.013	4.756	4
B3	0.61	0.03	4.27	4
B4	1.51	0.05	3.00	4

F. Recovery:

When samples are spiked with known concentrations of human ACE2, the recovery averages range from 93% to 112%.

G. Linearity:

Different samples containing human ACE2 were diluted several fold(1/2 to1/8 for sera and plasmas) and the measured recoveries ranged from 90% to 111%.

H. Expected values:

Human ACE2 protein levels range in serum and plasma from **Non Detectable (ND) to > 2ng/ml**.

Human ACE2 protein levels range in urine from ~2ng/ml to > 16ng/ml**.**

12. Technical Hints and Limitations

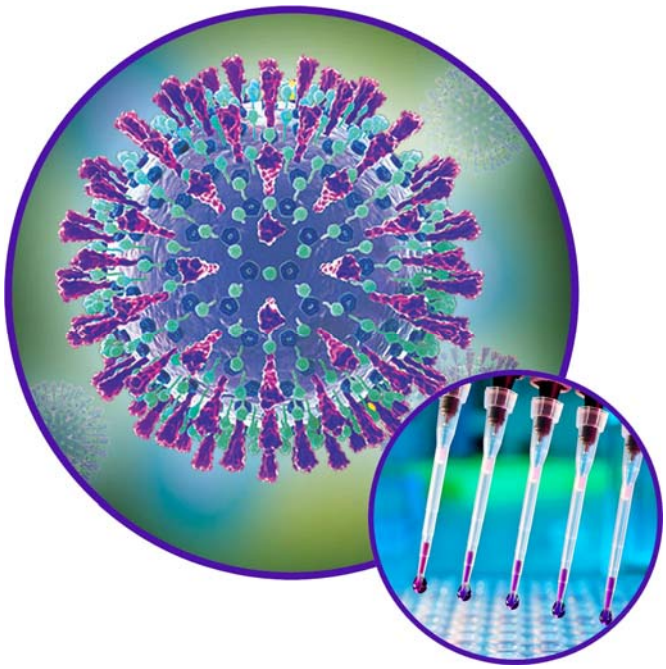
- It is recommended that all standards and samples be run in duplicate.
- Do not combine left over reagents with those reserved for additional wells.
- Reagents from the kit with a volume less than100µl should be centrifuged.
- Residual wash liquid should be drained from the wells after last wash by tapping the plate on absorbent paper.
- Crystals could appear in the10X solution due to high salt concentration in the stock solutions. Crystals are readily dissolved at room temperature or at 37°C before dilution of the buffer solutions.
- Once reagents have been added to the16-well strips, DO NOT let the strips DRY at anytime during the assay.
- Keep TMB Solution protected from light.
- The Stop Solution (STOP) consists of sulfuric acid. Although diluted, the Stop Solution should be handled with gloves, eye protection and protective clothing.

13. Troubleshooting

PROBLEM	POSSIBLE CAUSES	SOLUTIONS
No signal or weak signal	Omission of key reagent	Check that all reagents have been added in the correct order.
	Washes too stringent	Use an automated plate washer if possible.
	Incubation times in adequate	Incubation times should be followed as indicated in the manual.
	Plate reader settings not optimal	Verify the wavelength and filter setting in the plate reader.
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrates to room temperature before use.
High background	Concentration of STREP-HRP too high	Use recommended dilution factor.
	Inadequate washing	Ensure all wells are filling wash buffer and are aspirated completely.
Poor standard curve	Wells not completely aspirated	Completely aspirate wells between steps.
	Reagents poorly mixed	Be sure that reagents are thoroughly mixed.
Unexpected results	Omission of reagents	Be sure that reagents were prepared correctly and added in the correct order.
	Dilution error	Check pipetting technique and double-check calculations.

14. Notes

1. Y.Sakaki,etal.; J.Med.Invest. **68,** 292(2021)
2. A.Lundström,etal.; J.Med.Virol. **93,**5908(2021)



ABEOMICS, Inc.
9853 Pacific Heights Blvd, STE D.
San Diego, CA-92121
Tel: 858-263-4982
Fax: 858-247-7052
Email: info@abeomics.com
Web site: www.abeomics.com

abeomics
Antibodies & Engineered Cell Lines™