Version 22-04/24

User's Manual

Human ACE2 ELISA Kit

REF **DEIA4483**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

The Human ACE2 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.

General Description

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. 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. ACE2 shares approximately 60% homology with ACE, the other key enzyme of the RAS system. ACE2 converts angiotensin II (Ang II) into Ang, 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. 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. 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. 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. Soluble ACE2 might also be used as biomarker of hypertension and cardiovascular diseases.

Principles of Testing

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 precoated onto the 96-well microtiter plate. Standards (STD) and samples are pipetted 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.

Reagents And Materials Provided

Coated with ACE2 Antibody, 6×16-well strips, 1 Plate

Wash Buffer (10X), 2×30 ml, 2 bottles

Sample Buffer, 5×30 ml, 1 bottle

Detection Antibody, 0.02 ml, 1 vial

HRP Labeled Streptavidin, lyophilized, 1 vial

Human ACE2 Standard, lyophilized, 1 vial

TMB Substrate Solution, 12 ml, 1 bottle

Stop Solution, 12 ml, 1 bottle

Plate sealers (plastic film), ×2

Materials Required But Not Supplied

1. Microtiterplate reader at 450 nm
2. Calibrated precision pipettes. Disposable pipette tips
3. Deionized water
4. Microtubes or equivalent for preparing dilutions
5. Disposable plastic containers for preparing working buffers
6. Plate washer: automated or manual
7. Glass or plastic tubes for diluting and aliquoting standard

Storage

The kit should be stored at 2-8°C. Under these conditions, all components are stable until the expiration date (see label on the box).

After standard reconstitution, prepare aliquots and store at -20°C. Avoid freeze/thaw cycles. Plate and reagents should reach room temperature before use.

Specimen Collection And Preparation

Serum: Use a serum separator tube. Let samples clot at room temperature for 30 minutes before centrifugation for 20 minutes 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 Buffer 1X. Samples containing visible precipitates must be clarified before use.

NOTE: As a starting point, 1/2 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!

Reconstitution And Storage

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.

The reconstituted standard is aliquoted and stored at -20°C!

Reagent Preparation

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

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

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

4. **HRP Labeled Streptavidin (STREP-HRP)** has to be reconstituted with 100 µl of Sample Buffer 1×.

   a. After reconstitution of STREP-HRP, prepare aliquots and store them at -20°C. Avoid freeze/thaw cycles.

   b. Dilute the reconstituted STREP-HRP to the working concentration by adding 50 µl in 10 ml of Sample Buffer 1× (1:200).

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

5. **Human ACE2 Standard (STD)** has to be reconstituted with 100 µl of Sample Buffer 1×.

   a. 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: The reconstituted standard is aliquoted and stored at -20°C!**

   b. Dilute the standard protein concentrate (STD) (1 µg/ml) in Sample Buffer 1×. A sevenpoint standard curve using 2-fold serial dilutions in Sample Buffer 1× is recommended.

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

6. **Start with the dilution of the concentrate (STD):**

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

7. **Dilute further for the standard curve:**

To obtain	Add	Into
4 ng/ml	240 µl of ACE2 (10 ng/ml)	360 µl of Sample Buffer 1X
2 ng/ml	300 µl of ACE2 (4 ng/ml)	300 µl of Sample Buffer 1X
1 ng/ml	300 µl of ACE2 (2 ng/ml)	300 µl of Sample Buffer 1X
0.5 ng/ml	300 µl of ACE2 (1 ng/ml)	300 µl of Sample Buffer 1X
0.25 ng/ml	300 µl of ACE2 (0.5 ng/ml)	300 µl of Sample Buffer 1X
0.125 ng/ml	300 µl of ACE2 (0.25 ng/ml)	300 µl of Sample Buffer 1X
0.0625 ng/ml	300 µl of ACE2 (0.125 ng/ml)	300 µl of Sample Buffer1X
0 ng/ml	300 µl of Sample Buffer 1X	Empty tube

Assay Procedure

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 minibags and stored at 4°C.

NOTE: Remaining 16-well strips coated with ACE2 antibody when opened can be store 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 (refer to the sections **Reagents Preparation** and **Specimen Collection And Preparation**).
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 1× 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. Add 100 µl to each well of the diluted Detection Antibody (DET).
6. Cover the plate with plastic film and incubate for **1 hour at Room Temperature**.
7. Aspirate the coated wells and add 300 µl of Wash Buffer 1× 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. Add 100 µl to each well of the diluted HRP Labeled Streptavidin (STREP-HRP) (refer to the sections **Reagents Preparation**).
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 1× 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 100 µl to each well of TMB Substrate Solution.
12. Allow the color reaction to develop **at room temperature (RT°C) in the dark for 20-25 minutes**. Do not cover the plate.

13. Stop the reaction by adding 100 µl of Stop Solution. Tap the plate gently to ensure thorough mixing. The substrate reaction yields a blue solution that turns yellow when Stop Solution is added.

CAUTION: CORROSIVE SOLUTION!

14. Measure the OD at 450 nm in an ELISA reader.

Calculation

1. Average the duplicate readings for each standard and sample and subtract the average blank value (obtained with the 0 ng/ml point).
2. 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 (Y) axis.
3. Calculate the ACE2 concentrations of samples by interpolation of the regression curve formula as shown above in a form of a quadratic equation.
4. If the test samples was diluted, multiply the interpolated values by the dilution factor to calculate the concentration of human ACE2 in the sample.

Typical Standard Curve

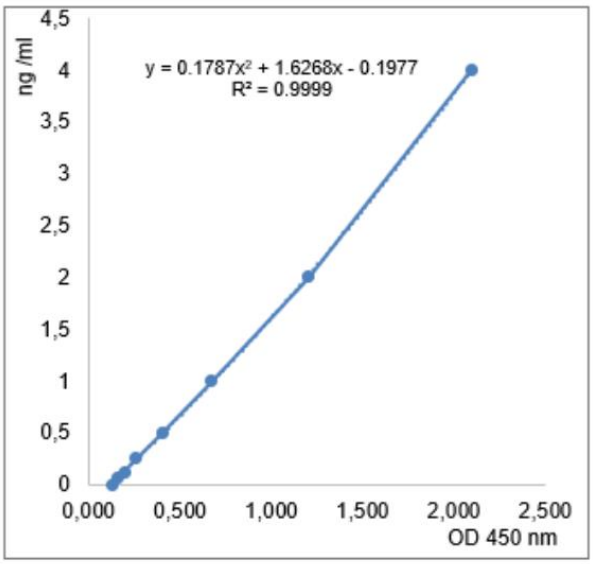

Figure: Standard curve

Precision

1. **Intra-assay precision:**

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

Sample	Mean (ng/ml)	SD	CV (%)	n
A1	2.02	0.04	2.05	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

2. **Inter-assay precision:**

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

Sample	Mean (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

Detection Range

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>16 ng/ml

0. 625-4 ng/ml

Detection Limit

40 pg/ml

Specificity

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

Linearity

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

Recovery

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

Precautions

1. It is recommended that all standards and samples be run in duplicate.

2. Do not combine leftover reagents with those reserved for additional wells.
3. Reagents from the kit with a volume less than 100 µl should be centrifuged.
4. Residual wash liquid should be drained from the wells after last wash by tapping the plate on absorbent paper.
5. Crystals could appear in the 10× 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.
6. Once reagents have been added to the 16-well strips, **DO NOT** let the strips DRY at any time during the assay.
7. Keep TMB Substrate Solution protected from light.
8. The Stop Solution consists of sulfuric acid. Although diluted, the Stop Solution should be handled with gloves, eye protection and protective clothing.

Possible Problems and Interference Elimination

1. **No signal or weak signal**

   a. Omission of key reagent

   Check that all reagents have been added in the correct order.

   b. Washes too stringent

   Use an automated plate washer if possible.

   c. Incubation times inadequate

   Incubation times should be followed as indicated in the manual.

   d. Plate reader settings not optimal

   Verify the wavelength and filter setting in the plate reader.

   e. Incorrect assay temperature

   Use recommended incubation temperature. Bring substrates to room temperature before use.

2. **High background**

   a. Concentration of STREP HRP too high

   Use recommended dilution factor.

   b. Inadequate washing

   Ensure all wells are filling wash buffer and are aspirated completely.

3. **Poor standard curve**

   a. Wells not completely aspirated

   Completely aspirate wells between steps.

   b. Reagents poorly mixed

   Be sure that reagents are thoroughly mixed.

4. **Unexpected results**

   a. Omission of reagents

   Be sure that reagents were prepared correctly and added in the correct order.

b. Dilution error

Check pipetting technique and double-check calculations.

Tel: 1-631-624-4882 (USA)
Tel: 44-161-818-6441 (Europe)
Fax: 1-631-938-8221
Email: info@creative-diagnostics.com