HIGH SENSITIVITY HUMAN SOLUBLE ANGIOTENSIN-CONVERTING ENZYME 2 (ACE2) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN SOLUBLE ACE2 CONCENTRATIONS IN SERUM, PLASMA SALIVA AND URINE

ALWAYS REFER TO LOT SPECIFIC PROTOCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ AND CHECK ALL ITEMS OF EACK KIT BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

PRODUCT INFORMATION:
THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	**High Sensitivity Soluble ACE2 (Human) ELISA Kit**
Catalog No.	SK00707-01
Lot No.	
Formulation	96 T
Standard range	100 ~ 6400 pg/mL
Sensitivity	20 pg/mL
Sample Volume	100 µL
Dilution Factor	Optimal dilutions should be determined by each laboratory for each application.
Sample Type	Serum, EDTA plasma, Saliva, Urine
Specificity	Human Soluble ACE2
Calibration	The human sACE2 recombinant (HEK293)
Intra-assay Precision	4 - 8%
Inter-assay Precision	4 - 9%
Storage	2 - 8° C for 4 months. See page 2 for detail
This kit contains sufficient materials to run 35-40 samples duplicated provided that assay is run according to protocol.	

Order Contact:
AVISCERA BIOSCIENCE, INC
2348 Walsh Ave., Suite C
Santa Clara, CA 95051
USA
Tel: (408) 982 0300
Email: Info@AvisceraBioscience.com
Website: www.AvisceraBioscience.com
Website: www.AvisceraBioscience.net

INTRODUCTION

High Sensitivity Human Soluble Angiotensin-Converting Enzyme 2 (ACE2) immunoassay is a solid phase ELISA designed to measure human sACE2 in serum, EDTA plasma, saliva and urine. It contains recombinant the glycosylated human soluble ACE2 derived from HEK293 animal free and antibodies raised against this protein. It has been shown to accurately quantify recombinant human soluble ACE2. Results obtained with naturally occurring soluble ACE2 samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural human Soluble ACE2.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for ACE2 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any ACE2 present is bound by the immobilized antibody. After washing away any unbound substances, an antibody biotinylated specific for ACE2 is added to the wells. Following a wash to remove any unbound antibody, Streptavidin-HRP conjugate is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of ACE2 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.

_ It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

_ If samples generate values higher than the highest standard, dilute the samples with the appropriate Dilution Buffer 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 immunoassay, the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

Description	Code	Quantity
ACE2 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a purified Antibody against human ACE2.	**707-01-01**	**1 plate**
ACE2 Standard –12.8 ng per vial of recombinant glycosylated human sACE2 His Tag (HEK293) in a buffered protein base with preservative; lyophilized.	**707-01-02**	**1 vial**
Detection Antibody Concentrate – 1.2 mL per vial, 10-fold concentrate of purified antibody human ACE2 biotinylated with preservative; lyophilized.	**707-01-03**	**1 vial**
Positive Control – one vial of recombinant glycosylated human sACE2 His Tag (HEK293); lyophilized.	**707-01-04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µl/vial, 100-fold concentrated solution of SAHRP conjugate with preservative.	**SAHRP**	**1 vial**
Dilution Buffer – 45 mL of buffered protein based solution with preservative.	**DB10**	**1 bottle**
Antibody Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB0208C**	**1 bottle**
HRP Diluent Buffer – 12 mL of buffered protein based solution with preservative.	**DB08B**	**1 bottle**
Wash Buffer 20X- 25 mL of 20-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution – 11 mL of 0.25M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1**

Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 4 months. For longer storage for up to 10 months, unopened Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer and HRP Diluent Solution should be stored at -20° C. Streptavidin-HRP Conjugate and TMB Substrate Solution should be stored only at 2 -8 °C. Do not use kit past expiration date.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

SAMPLE COLLECTION AND STORAGE

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 ≤ -20° C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA 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.

Saliva – Collect 6 ~7 ml of the saliva samples in 15 ml or 50 ml centrifuge tube. Immediately store samples at ice bath. Centrifuge it for 20 minutes at 4000 rpm at 2- 8 °C. Remove 5 ~ 6 ml of the clear part of saliva samples without any pellets and store the saliva samples at -70° C.

5 ~ 6 ml of each saliva sample need 15 ~ 20 fold concentrated and washed with 1 x PBS on Pierce Protein Concentrator MWCO, 10K, 5-20 mL (Thermo Scientific REF: 88528). Centrifuge it for 2 hours at 4000 rpm at 2 ~ 8 °C.

Finally collect the concentrated solution at 250 ~ 300 µL (concentrated factor as 20) and stored at – 70 °C for ACE2 assay.

If saliva samples have been concentrated by 20 fold, the concentration read from the standard curve must be divided by the concentrated factor 20.

SAMPLE PREPARATION

Serum or plasma samples may require dilution.
Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 25 mL of Wash Buffer Concentrate into deionized or distilled water (475 mL) to prepare 500 mL of 1x Wash Buffer.

Human ACE2 Standard - Reconstitute the Human ACE2 standard with 1 mL of Dilution Buffer. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Dilution Buffer into tubes #2 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **6400 pg/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL). Reconstituted ACE2 standard stock solution can be stored at – 20 ~ - 70 °C for a few days.

Tube	Standard	Dilution Buffer	Concentration
stock	powder	1 mL	12800 pg/mL
# 1	250 µl of stock	250µl	6400 pg/ml
# 2	250µl of 1	250µl	3200 pg/ml
# 3	250µl of 2	250µl	1600 pg/ml
# 4	250µl of 3	250µl	800 pg/ml
# 5	250µl of 4	250µl	400 pg/ml
# 6	250µl of 5	250µl	200 pg/ml
# 7	250µl of 6	250µl	100 pg/ml

Positive Control - Reconstitute the Positive Control with 1 mL of Dilution Buffer to prepare 1 x working solution. *Discard the 1 x working solution after use.*

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.2 ml of **Antibody Diluent Solution (DB0208C)** to produce a 10-fold concentrated stock solution. For 96 wells test, freshly pipette 9.45 mL of **Antibody Diluent Solution (DB0208C)** into a 15 mL centrifuge tube and

transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution. For run a partial strip test, freshly prepare 900µL of working solution per 8-well strip.

Streptavidin-HRP Conjugate – For 96 wells test, freshly pipette 11.88 mL of **HRP Diluent Buffer (DB08B)** into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution Streptavidin-HRP conjugate (**protect from light**) should be used within 20 min.

For run a partial strip test, freshly prepare 900µL of working solution per 8-well strip.
Always store the 100-fold concentrated streptavidin HRP conjugate at 2 ~ 8 °C for 8 months.

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Add 100 µL of Dilution Buffer to Blank wells.
3. Add 100 µL of standard dilutions in reverse order of serial dilution, samples, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 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.
5. Add 100 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hour on microplate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of Streptavidin-HRP conjugate working solution to each well. Incubate for 60 minutes on microplate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of Substrate Solution to each well. Incubate for 15 ~ 20 minutes on microplate shaker at room temperature. **Protect from light.**
10. Add 100 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 if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 3 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive 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 log-log or 4-parameter curve fit.

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

If saliva samples have been concentrated, the concentration read from the standard curve must be divided by the concentrated factor.

TYPICAL DATA

The standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

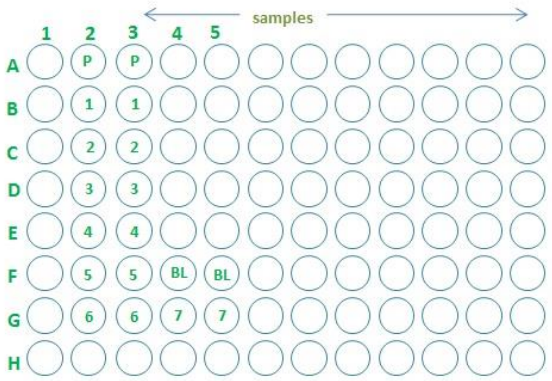
STANDARD (PG/ML)	AVERAGE OD450NM (CORRECTED)*
Blank	0 (0.086)
100	0.030
200	0.069
400	0.129
800	0.261
1600	0.525
3200	1.109
6400	2.019

Lot No.: 20114496
Positive control: 1 x solution: 600 ~2700 pg/mL

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human ACE2 (HEK293)	100
Human ACE2/Fc Fusion (HEK293)	100
Human ACE1 (HEK293)	0
Human ACTR-2A/ Fc (HEK293)	0
Human CD87 (HEK293)	0
Human DPPIV (HEK293)	0
Human Renin (HEK293)	0
Human Neprilysin (HEK293)	0

Human soluble ACE2 recombinant derived from *E. Coli* or sf21 may not be detected by this ELISA kit.



SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µL of standard dilutions, samples, or positive control each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL Detection Antibody working solution to each well. Incubate 2 hour on the plate shaker at RT.
↓
Add 100 µL Streptavidin-HRP conjugate working solution to each well. Incubate 60 min on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µL Substrate Solution to each well. Incubate 15 ~ 20 min on plate shaker at RT. **Protect from light.**
↓
Add 100 µL Stop Solution to each well. Read 450nm within 3 min.