

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

FOR THE QUANTITATIVE DETERMINATION  
OF HUMAN SOLUBLE ACE2  
CONCENTRATIONS IN SERUM, PLASMA  
AND CELL CULTURES



ALWAYS REFER TO LOT SPECIFIC PROTOCOL  
PROVIDED WITH EACH KIT FOR  
INSTRUCTIONS. PROTOCOL MUST BE  
READ AND CHECK ALL ITEMS OF EACH 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	Human Soluble ACE2 ELISA Kit
Catalog No.	SK00707-08B
Lot No.	
Formulation	2 x 96 T
Standard range	196 – 12500 pg/mL
Sensitivity	49 pg/mL
Sample Volume	100 µL of diluted samples
Dilution Factor	Optimal dilutions should be determined by each laboratory for each application.
Sample Type	Serum, EDTA plasma, Cell Cultures
Specificity	Human Soluble ACE2 and Human ACE2-Fc Fusion
Calibration	Glycosylated Human Soluble ACE2 (HEK293)
Intra-assay Precision	4 - 8%
Inter-assay Precision	8 - 12%
Storage	2 - 8° C for 1 month. See page 2 for detail
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

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**INTRODUCTION**

Human Soluble Angiotensin-Converting Enzyme 2 (ACE2) immunoassay is a solid phase ELISA designed to measure human sACE2 in serum, EDTA plasma, saliva and cell cultures. It contains recombinant human soluble ACE2 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
<b>ACE2 Microplate</b> - 96 well polystyrene microplate (12 strips of 8 wells) coated with a purified Antibody against human soluble ACE2.	<b>707-08-01</b>	<b>1 plate</b>
<b>ACE2 Standard</b> – 50 ng/vial of recombinant human sACE2 in a buffered protein base with preservative; lyophilized.	<b>707-08-02</b>	<b>1 vial</b>
<b>Detection Antibody Concentrate</b> – 1.05 mL per vial, 10-fold concentrate of purified antibody biotinylated against human ACE2 with preservative; lyophilized.	<b>707-08-03</b>	<b>1 vial</b>
<b>Positive Control</b> – one vial of recombinant human ACE2; lyophilized.	<b>707-08-04</b>	<b>1 vial</b>
<b>Streptavidin-HRP Conjugate</b> - 120 µl/vial, 100-fold concentrated solution of SAHRP conjugate with preservative.	<b>SAHRP</b>	<b>1 vial</b>
<b>Dilution Buffer</b> – 45 mL of buffered protein based solution with preservative.	<b>DB10</b>	<b>1 bottle</b>
<b>Antibody Diluent Solution</b> – 12 mL of buffered protein based solution with preservative.	<b>DB108F</b>	<b>1 bottle</b>
<b>HRP Diluent Buffer</b> – 12 mL of buffered protein based solution with preservative.	<b>DB08C</b>	<b>1 bottle</b>
<b>Wash Buffer</b> - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	<b>WB01</b>	<b>1 bottle</b>
<b>TMB Substrate Solution</b> - 11 mL of TMB substrate solution.	<b>TMB01</b>	<b>1 bottle</b>
<b>Stop Solution</b> – 11 mL of 0.5M HCl.	<b>S-STOP</b>	<b>1 bottle</b>
<b>Plate Sealer</b>	<b>EAPS</b>	<b>1</b>

Plastic Pouch	P01	1
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**STORAGE**

**Unopened Kit:** Store at 2 - 8° C for up to 1 month. 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 (350-400rpm).
- 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.

**SAMPLE PREPARATION**

EDTA Plasma samples may require 20- 40 fold dilution. Serum samples may need 16 ~ 32 fold 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 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

**Human ACE2 Standard** - Reconstitute the Human ACE2 standard with 1ml 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 #1 to #8. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **25000 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	50000 pg/ml
# 1	150µl of stock	450µl	12500 pg/ml
# 2	250µl of 1	250µl	6250 pg/ml
# 3	250µl of 2	250µl	3125 pg/ml
# 4	250µl of 3	250µl	1560 pg/ml
# 5	250µl of 4	250µl	780 pg/ml
# 6	250µl of 5	250µl	390 pg/ml
# 7	250µl of 6	250µl	195 pg/ml

**Positive Control** - Reconstitute the Positive Control with 2 mL of Dilution Buffer.

**Detection Antibody Concentrate** - Reconstitute the Detection Antibody Concentrate with 1.05 ml of **Antibody Diluent Solution (DB108F)** to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of **Antibody Diluent Solution (DB108F)** into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution. **Note:** *Detection Antibody Concentrate and its working solution CAN NOT USE Dilution Buffer (DB10) or HRP Diluent Buffer (DB08C) as dilution matrix.*

**Streptavidin-HRP Conjugate** - Pipette 11.88 mL of **HRP Diluent Buffer (DB08C)** 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 a few hours.

**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 17-22 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.

**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 (0.139)
195	0.036
390	0.073
780	0.144
1560	0.309
3125	0.457
6250	0.891
12500	1.591

Positive Control: 1 ~ 6 ng/mL








**SPECIFICITY**

PROTEINS	CROSS-REACTIVITY (%)
Human ACE2 His Tag (HEK293)	100
Human ACE2/Fc Fusion (HEK293)	100
Human ACE1 (HEK293)	0
Human CD87 (HEK293)	0
Human DPPIV (HEK293)	0
Human Renin (HEK293)	0
Human Nephilysin (HEK293)	0
RBD Spike Protein (SARS-CoV-2) His Tag (HEK293)	0
RBD Spike Protein (SARS-CoV-2) –mFc Fusion (HEK293)	0
S1 Spike Protein (SARS-CoV-2) His Tag (HEK293)	0

Human ACE2 recombinant derived from E. Coli or sf21 cells may NOT be detected by this ELISA Kit.

**SUMMARY OF ASSAY PROCEDURE**

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PREPARE REAGENTS, SAMPLES AND STANDARDS

Add 100 $\mu$ L 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 $\mu$ L Detection Antibody working solution to each well. Incubate 2 hour on the plate shaker at RT.

Add 100 $\mu$ L Streptavidin-HRP conjugate working solution to each well. Incubate 60 min on the plate shaker at RT. <b>Protect from light.</b>

Aspirate and wash 4 times.

Add 100 $\mu$ L Substrate Solution to each well. Incubate 17-22 on plate shaker at RT. <b>Protect from light.</b>

Add 100 $\mu$ L Stop Solution to each well. Read 450nm within 3 min.