# Human ACE2 ELISA Kit

**For detection and measurement of human ACE2**

Catalog #100-0687 1 Kit

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

## Product Description

The Human ACE2 ELISA Kit is designed for the quantitative detection and measurement of human angiotensin converting enzyme 2 (ACE2) in biological fluids such as serum, plasma, and cell culture supernatant.

ACE2 is a type I integral membrane protein. It is highly expressed in capillary-rich organs such as the lungs, heart, blood vessels, kidneys, liver, and gastrointestinal tract. The ACE2 receptor is the target of the SARS-CoV-2 Spike (S) glycoprotein. This interaction allows for viral entry into the host cell, making ACE2 a potential target for antiviral therapeutics.

The assay is based on the sandwich ELISA method, in which samples are added to ELISA strip plates pre-coated with capture antibodies specific for ACE2. The captured ACE2 is detected by addition of a biotinylated detection antibody, followed by streptavidin-horseradish peroxidase (SA-HRP), which binds the biotinylated antibody. Addition of the chromogenic enzyme substrate 3,3',5,5' tetramethylbenzidine (TMB) results in a colored product with an intensity directly proportional to the concentration of ACE2 in the sample. The concentration of ACE2 is determined by comparison to a serial dilution of the standard analyzed in parallel.

## Product Information

All components listed below are stable until expiry date (EXP) on label. Kit may be shipped at room temperature (15 - 25°C) but should be stored at 2 - 8°C upon receipt. Once components are opened, store at 2 - 8°C and use within 1 month.

| COMPONENT NAME | COMPONENT # | SIZE | STORAGE | DESCRIPTION |
|---|---|---|---|---|
| Human ACE2 Standard | 300-0318 | 2 vials | Store at 2 - 8°C. | Lyophilized recombinant human ACE2 |
| Human ACE2 ELISA Plate | 300-0317 | 1 plate | Store at 2 - 8°C. | Plate (12 strips x 8 wells) coated with anti-human ACE2 antibody |
| Human ACE2 Biotinylated Detection Antibody | 300-0319 | 120 µL | Store at 2 - 8°C. | Biotinylated anti-human ACE2 antibody (100X) |
| Human ACE2 Antibody Diluent | 300-0321 | 15 mL | Store at 2 - 8°C. | For dilution of detection antibody |
| Adhesive Plate Covers | 300-0327 | 4 covers | Store at 2 - 8°C. | For covering plates during incubation |
| Stop Solution* | 300-0326 | 10 mL | Store at 2 - 8°C. | 4 N Sulfuric acid |
| TMB Substrate | 300-0325 | 10 mL | Store at 2 - 8°C. | 3,3',5,5' tetramethylbenzidine (TMB) enzyme substrate solution |
| ELISA Diluent | 300-0323 | 50 mL | Store at 2 - 8°C. | Buffer for dilution of standard and samples |
| SA-HRP | 300-0320 | 120 µL | Store at 2 - 8°C. | Streptavidin-horseradish peroxidase (SA-HRP) conjugate |
| SA-HRP Diluent | 300-0322 | 15 mL | Store at 2 - 8°C. | For dilution of SA-HRP |
| Wash Buffer (25X) | 300-0324 | 20 mL | Store at 2 - 8°C. | Concentrated buffer solution for washing plates |

*Please refer to the Safety Data Sheet (SDS) for hazard information.

## Materials Required But Not Included

- Biohazard safety cabinet certified for Level II handling of biological materials
- Microplate reader set at a wavelength of 450 nm and (optional) a second correction wavelength set at 540 nm or 570 nm
- 37°C incubator
- ELISA plate washer: Automatic (adaptable for ELISA strip plates) or manual (e.g. multi-pipette or squirt bottle)
- Pipettor with appropriate tips
- Beakers, flasks, and graduated cylinders necessary for reagent preparations
- Tubes for standard and sample dilutions
- Timer
- Absorbent paper
- Deionized or distilled water

## Preparation of Reagents and Materials

### A. Wash Buffer

NOTE: Use clean or disposable glass or plasticware for preparation and storage of wash buffer.

NOTE: If crystals are visible in the concentrate, warm to room temperature and mix gently until completely dissolved.

Dilute Wash Buffer (25X) 1 in 25 with distilled or deionized water.

*Example: For one plate, prepare 500 mL wash buffer (1X) by adding 20 mL Wash Buffer (25X) to 480 mL distilled or deionized water.*

### B. ELISA Plate

1. Allow plate to adjust to room temperature (15 - 25°C) before opening the bags.
2. Plan the experiment to include a standard curve and background control (8 x 2 wells), a blank (2 wells), and test samples.
3. Assemble the required number of strips in the plate frame. Store the remaining strips in the foil bag containing the desiccant at 2 - 8°C for up to 1 month.

### C. Standard Stock Solution

NOTE: It is recommended to prepare fresh standard stock solution for each assay and use within 4 hours. However, reconstituted standard stock solution can be stored for 1 week at 2 - 8°C.

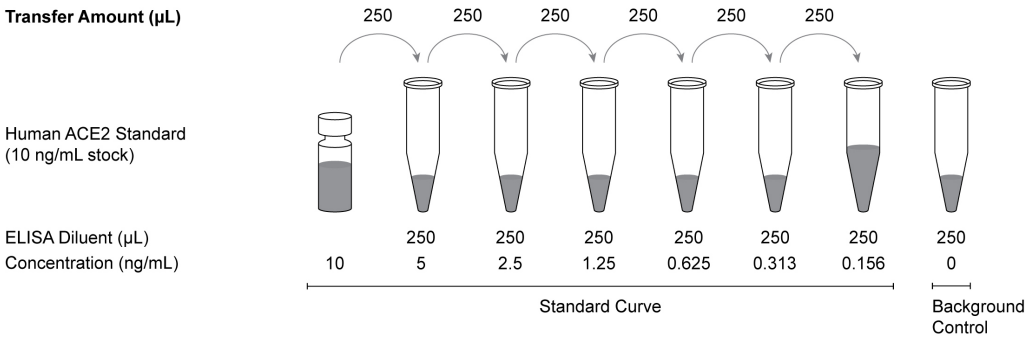
1. Centrifuge the standard vial to bring all material to the bottom of the vial.
2. Add 1 mL of ELISA Diluent to the vial of Human ACE2 Standard (final concentration 10 ng/mL). Mix gently to ensure complete reconstitution (avoid foaming) and let sit for a minimum of 15 minutes.
3. Mix thoroughly.

### D. Standard Curve

NOTE: The standard curve dilutions should be prepared from standard stock solution and used within 1 hour. Duplicate wells for the standard curve dilutions and the background control are recommended.

Dilute the standard stock solution (prepared in section C) to create standard curve dilutions ranging from 0.156 - 10 ng/mL according to Figure 1.

For the background control (0 pg/mL), use only ELISA Diluent.




NOTE: Volumes indicated are sufficient for duplicates.

**Figure 1. Recommended Serial Dilution of Standard**

### E. Samples

Refer to instructions below for preparing serum, plasma, or cell culture supernatant. Avoid using lipemic, hemolyzed, or contaminated samples, as these may yield unreliable results. If necessary, dilute samples in ELISA Diluent; this dilution may need to be optimized for individual samples. Fresh samples are recommended, to reduce protein degradation and denaturation risk. However, samples to be used within 5 days can be stored at 2 - 8°C. For samples to be used within 1 month, store at -20°C; for storage for 1 - 2 months, store samples at -80°C to avoid loss of bioactivity and contamination.

NOTE: Duplicate wells for all samples are recommended. For samples with a high protein concentration, it is advisable to prepare several dilutions for testing. Remove any particulate matter by centrifugation or filtration prior to use.

Serum

1. Using a serum separator tube (SST), allow samples to clot at room temperature (15 - 25°C) for 2 hours or overnight at 2 - 8°C.
2. Centrifuge at 1000 x *g* for 15 minutes. Remove serum fraction and transfer to a fresh tube.

   NOTE: If not used immediately, aliquot and store at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Plasma

1. Use EDTA or heparin as an anticoagulant when collecting plasma.
2. Within 30 minutes of collection, centrifuge plasma at 1000 x *g* for 15 minutes at 2 - 8°C. Remove and discard serum fraction, then transfer plasma to a fresh tube.

   NOTE: If not used immediately, aliquot and store at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Cell Culture Supernatant

Centrifuge cell culture supernatant at 1000 x *g* for 15 minutes to remove particulates. Transfer supernatant to a fresh tube.

NOTE: If not used immediately, aliquot and store at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

### F. Detection Antibody

1. Centrifuge the vial of Human ACE2 Biotinylated Detection Antibody to bring all material to the bottom of the vial.
2. Dilute Human ACE2 Biotinylated Detection Antibody 1 in 100 in Human ACE2 Antibody Diluent.

   *Example: Add 10 µL of Human ACE2 Biotinylated Detection Antibody to 990 µL of Human ACE2 Antibody Diluent.*

### G. SA-HRP

Dilute SA-HRP 1 in 100 in SA-HRP Diluent.

*Example: Add 10 µL SA-HRP to 990 µL SA-HRP Diluent.*

## Directions for Use

Please read the entire protocol before proceeding. For each step in the protocol, total dispensing time for the addition of reagents or samples to the plate should not exceed 10 minutes. In all washing steps, each well must be thoroughly washed by filling with wash buffer, allowing to sit for 2 minutes, then blotting dry by tapping the plate upside down on absorbent paper.

NOTE: Cross-contamination of reagents may invalidate assay results. Permanently labeled, dedicated, multi-channel micropipette reservoirs for reagents are recommended.

1. Centrifuge thawed samples to bring all material to the bottom of the vial. Bring all reagents and samples to room temperature (15 - 25°C), except the TMB Substrate, which should be kept at 2 - 8°C until use.
2. Prepare wash buffer (1X), reagents, samples, standard dilutions, and the ELISA plate as described in Preparation of Reagents and Materials.
3. Add 100 µL/well of each standard curve dilution and background control to ELISA plate. Leave the blank wells empty.
4. Add 100 µL/well of diluted test samples to ELISA plate.

   NOTE: Duplicate wells for all samples are recommended.
5. Cover the plate with an Adhesive Plate Cover and incubate at 37°C for 2 hours.
6. Remove the liquid in each well and blot dry. **Do not wash the wells.**
7. Add 100 µL/well of Detection Antibody. Leave blank wells empty.

   NOTE: If Detection Antibody appears cloudy, warm to room temperature and mix gently until solution appears uniform.
8. Cover the plate with an Adhesive Plate Cover and incubate at 37°C for 1 hour.
9. Wash each well with 5 x 200 µL of wash buffer and blot dry.
10. Add 100 µL/well of diluted SA-HRP. Leave blank wells empty.
11. Cover the plate with an Adhesive Plate Cover and incubate at 37°C for 1 hour.

12. Wash each well with 5 x 200 µL of wash buffer and blot dry.
13. Add 90 µL/well of TMB Substrate to all wells (including blank wells).
    NOTE: TMB Substrate should be colorless or light blue until added to the plate. Protect TMB Substrate from light.
14. Incubate at 37°C in the dark for 15 - 30 minutes.
15. Add 50 µL/well of Stop Solution to all wells (including blank wells), in the same order as TMB Substrate addition. Gently tap the plate to ensure thorough mixing.
    NOTE: Use care when handling Stop Solution. Please refer to the Safety Data Sheet (SDS) for hazard information.
    NOTE: The color in the wells will turn from blue to yellow after addition of Stop Solution. Wells that are green indicate they are not thoroughly mixed.
16. Within 5 minutes of adding Stop Solution, measure the absorbance at 450 nm in a microplate reader. If possible, use a correction wavelength of 540 nm or 570 nm. Blank the reader using ELISA wells containing TMB Substrate and Stop Solution only.
    NOTE: Before measuring absorbance, remove any air bubbles in the wells using a small hypodermic needle or a pipette tip.
17. Calculate the average absorbance of each sample from the duplicate values. Subtract the mean absorbance value of the blank from the standard, the background control, and the sample values prior to generating the standard curve and determining the protein concentrations in the samples. A representative standard curve is shown in Figure 2.
    NOTE: Multiply the protein concentrations by the dilution factor used for each sample.

## Performance of the Assay

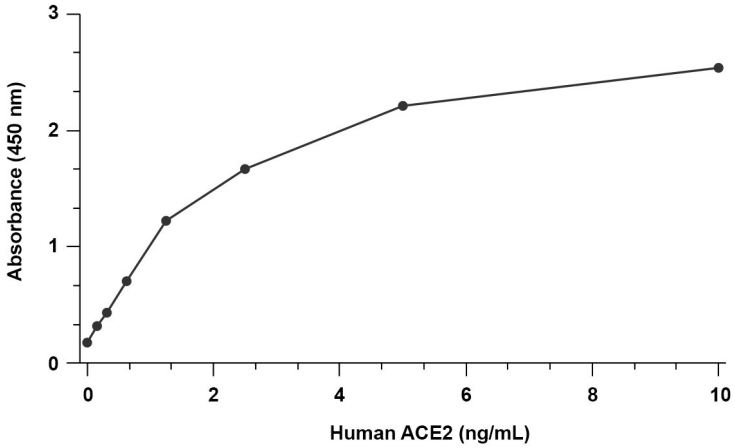



**Figure 2. Representative Standard Curve**

- Reportable range: 0.156 - 10 ng/mL. This is the concentration range in which measurement of the analyte can be done with the highest precision, accuracy, and linearity.
- Sensitivity: The lower limit of detection (LLD) of this assay is 39 pg/mL. This is the lowest protein concentration that could be differentiated from zero as determined by the mean OD value of 20 replicates of the zero standard added by their three standard deviations.
- Precision: The intra-assay precision (CV) of this assay is < 8%. The inter-assay precision (CV) of this assay is < 10%.
- Specificity: This assay has high specificity for the detection of human ACE2. No significant cross-reactivity or interference is known to occur between human ACE2 and analogues.

# Troubleshooting

| PROBLEM | POSSIBLE CAUSE | RECOMMENDED ACTION |
|---|---|---|
| Absorbance values too low | Incubation time too short | Follow the directions for the multiple incubation steps outlined in Directions for Use. |
| | Undiluted Wash Buffer (25X) | Dilute Wash Buffer (25X) with distilled or deionized water as described in Preparation of Reagents and Materials, section A. |
| | Incorrect dilution of Detection Antibody and/or SA-HRP | Follow the directions for dilution of Detection Antibody and SA-HRP in Preparation of Reagents and Materials. |
| | Wash step omitted before adding SA-HRP | Follow sequence of incubation and wash steps as outlined in Directions for Use. |
| Absorbance values too high | Incubation time too long | Follow the directions for the multiple incubation steps outlined in Directions for Use. |
| | Insufficient washing | Ensure that each well is washed 5X with 200 µL of diluted Wash Buffer. Ensure that the multi-channel pipette fills and empties reproducibly. Blot wells dry before proceeding to the next step.<br>If using an automated plate washer, add a 30-second soak period following the addition of wash buffer, and/or rotate the plate 180 degrees between wash steps. |
| | Incorrect dilution of Detection Antibody and/or SA-HRP | Follow the directions for dilution of Detection Antibody and SA-HRP in Preparation of Reagents and Materials. |
| Low absorbance readings despite good color development in wells | Incorrect wavelength setting on the ELISA reader | Check that the measuring wavelength is set at 450 nm and that the ELISA reader has the correct filter for this wavelength. |
| High background | Insufficient washing | Ensure that each well is washed 5X with 300 µL of diluted Wash Buffer. Ensure that the multi-channel pipette fills and empties reproducibly. Blot wells dry before proceeding to the next step. |
| | Incorrect dilution of Detection Antibody and/or SA-HRP | Follow the directions for dilution of Detection Antibody and SA-HRP in Preparation of Reagents and Materials. |
| No signal with standard curve dilutions | Reagents not added in correct sequence | Follow sequence of incubation and wash steps as outlined in Directions for Use. |
| | Incorrect dilution of Detection Antibody and/or SA-HRP | Follow the directions for dilution of Detection Antibody and SA-HRP in Preparation of Reagents and Materials. |
| Inconsistent dose-response curve | Incubation conditions for individual wells not identical | Avoid delays during filling of assay wells; ensure that all reagents are at 15 - 25°C prior to beginning the assay. |
| Poor replicates | Insufficient washing | Ensure that each well is washed 5X with 200 µL of diluted Wash Buffer. Ensure that the multi-channel pipette fills and empties reproducibly. Blot wells dry before proceeding to the next step.<br>If using an automated plate washer, add a 30-second soak period following the addition of wash buffer, and/or rotate the plate 180 degrees between wash steps. |
| | Unequal volumes in wells | Ensure that pipettes function properly. |
| | Carry-over between wells; evaporation from wells; splashing of well contents onto adhesive cover | Always use a new adhesive cover for each incubation. Ensure that each well is sealed tightly. Keep filled plates in a horizontal position and handle with caution. |
| | Samples not mixed after thawing | Vortex samples after thawing. |
| | High concentration of lipids or particulate matter in samples | Filter or centrifuge samples to pellet aggregates. Lipids may concentrate on the surface after centrifugation and may be removed. |
| | Air bubbles in well during measurement with the ELISA reader | Remove air bubbles using a small hypodermic needle or pipette tip. |
| Absorbance values of sample dilutions decrease with increasing concentration | Protein concentration of the sample dilutions exceeds the upper limit of the assay | Dilute the samples further and retest along with the standard curve dilutions. |
| Unexpected ELISA results for tissue or cell extraction samples | Certain chemicals present in the chemical lysis buffer | Try different lysis buffers |

## Related Products

For a complete list of ELISA kits and related products from STEMCELL Technologies, visit www.stemcell.com or contact us at techsupport@stemcell.com.