Human Angiotensin I Converting Enzyme 2 (ACE2) ELISA Kit

Catalog #: FM-E100236 (96 wells)

User Manual

This kit is designed to quantitatively detect the levels of human ACE2 in serum/ plasma, cell culture supernatants and other suitable sample solution.

Manufactured and Distributed by:

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Important notes

Before using this product, please read this manual carefully; after reading the subsequent contents of this manual, please note the following specially:

- The operation should be carried out in strict accordance with the provided instructions.
- Store the unused strips in a sealed foil bag at 2-8°C.
- Always avoid foaming when mixing or reconstituting protein solutions.
- Pipette reagents and samples into the center of each well, avoid bubbles.
- The samples should be transferred into the assay wells within 15 minutes of dilution.
- We recommend that all standards, testing samples are tested in duplicate.
- Using serial diluted sample is recommended for first test to get the best dilution factor.
- If the blue color develops too light after 15 minutes incubation with the substrate, it may be appropriate to extend the incubation time (Do not over-develop).
- Avoid cross-contamination by changing tips, using separate reservoirs for each reagent.
- Avoid using the suction head without extensive wash.
- Do not mix the reagents from different batches.
- Stop Solution should be added in the same order of the Substrate Solution.
- TMB developing agent is light-sensitive. Avoid prolonged exposure to the light.

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS.

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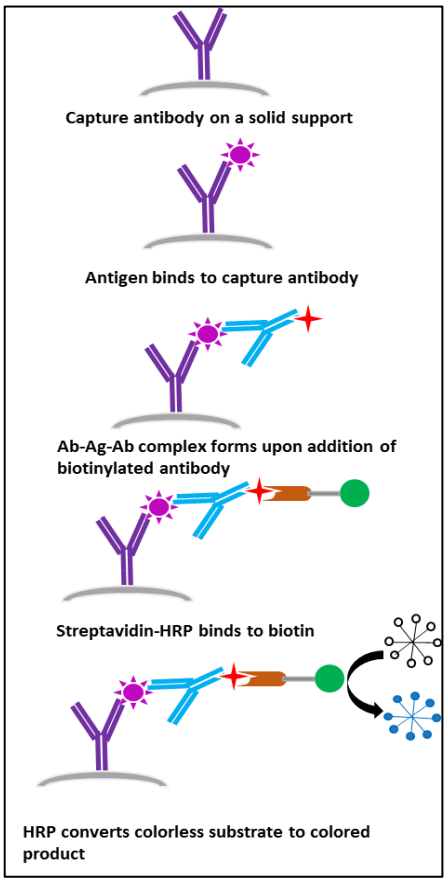
Intended use

The kit is used to quantify the human ACE2 in serum/ plasma, cell culture supernatant and other suitable sample solution.

Standard range	62.5 - 4,000 pg/mL
Assay time	3 h 40 min
Validity	Six months
Store at	2-8 °C

Assay principle

The Human ACE2 ELISA Kit is based on standard sandwich enzyme-linked immunosorbent assay technology. Anti-human ACE2 specific antibody has been pre-coated onto 96-well plate. Human ACE2 present in the standards/ samples bind to the capture antibody. Subsequently, biotinylated anti-human ACE2 detection antibody is added to form an Ab-Ag-Ab sandwich. After a washing step, streptavidin-HRP is added and the unbound conjugate is removed with wash buffer. Next, addition of HRP substrate, TMB, results in the production of a blue colored product that changes to yellow after the addition of acidic Stop Solution. The density of yellow color is directly proportional to the amount of human ACE2 captured on plate.



Materials supplied

1. Human ACE2 standard:	10 ng/vial ×2
2. 96-well plate pre-coated with anti-human ACE2 Ab:	1
3. Sample Diluent buffer :	12 ml × 2
4. Detection antibody:	1 vial, dilution 1:100
5. Streptavidin-HRP:	1 vial dilution 1:100
6. Antibody Diluent Buffer	12 ml
7. Streptavidin-HRP Diluent Buffer	12 ml
8. TMB Substrate (Ready to use):	10 ml
9. Stop Solution:	12 ml
10. 20 × Wash Buffer:	25 ml
11. Plate sealer	2
12. Package insert	1

Materials required but not supplied

- 1x PBS.
- Standard plate reader capable of measuring absorbance at 450 nm.
- Adjustable pipettes and disposable pipette tips.
- Multi-channel pipettes, manifold dispenser or automated microplate washer.
- Distilled water.
- Absorbent paper.
- Materials used for sample preparation.

Sample Preparation and storage

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

- Cell culture supernatant, tissue lysate or body fluids: Remove particulates by centrifugation, analyze immediately or aliquot and store at -20°C
- Serum: Allow the serum to clot in a serum separator tube (about 4hours) at room temperature. Centrifuge at approximately 1000 X g for 15 min. Analyze the serum immediately or aliquot and store frozen at -20°C.
- Plasma: Collect plasma using heparin as an anticoagulant. Centrifuge for 15 min at 1000 x g within 30 minutes of collection. Analyze immediately or aliquot and store frozen at -20°C. EDTA and citrate are not recommended as the anticoagulant.

Reagent Preparation

Preparation of standard solutions

- Human ACE2: Standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of standard (10 ng /vial) are included in each kit. Use one tube for each experiment.
- Prepare 4000 pg/ml→62.5 pg/ml of Human ACE2 standard solutions:
- Add 1 ml of Sample Diluent Buffer into one standard vial with 10 ng Human ACE2. Keep the tube at room temperature for 10 minutes and mix thoroughly. This is 10000 pg/ml standard solution. Add 0.4 ml of this solution to 0.6 ml of sample diluent buffer to prepare 4000 pg/ml standard solution.
- Label 6 Eppendorf tubes with 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, respectively. Then make 2-fold serial dilution from 4000 pg/ml to 62.5 pg/ml in 1.5 ml tubes with sample diluent buffer.
- Make sure each tube has ≥ 250 µl of standard.

Note: *The standard solutions are best used within 2 hours.*

Preparation of detection anti-human ACE2 antibody working solution

- The working solution should be prepared no more than 2 hours prior to the experiment
- The reagent is supplied as 100X concentrate. Empty the total contents in to 12.87 ml of Antibody Diluent Buffer or prepare the solution separately in a volume as needed. The solution should be mixed thoroughly.
- The total volume should be: 0.1 ml/well x the number of wells (Allowing 0.1-0.2 ml more than total volume.

Preparation of Streptavidin-HRP working solution

- The solution should be prepared no more than 1 hour prior to the experiment.
- The total volume should be: 0.1 ml/well x the number of wells (allowing 0.1-0.2 ml more than total volume).
- Streptavidin-HRP should be diluted 1:100 with Streptavidin-HRP Diluent buffer and mixed thoroughly.

Wash Buffer

- If crystals have formed in the 20X wash buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
- Dilute 25 ml Wash Buffer Concentrate (20X) to a total volume of 500 ml with distilled water.

Assay Procedure

Bring all reagents to room temperature before use. Human ACE2 standard curve should be prepared for each experiment. The user will decide sample dilution factor by rough estimation of Human ACE2 concentration in samples.

1. Add 100 µl of sample or standards per well. Add 0.1 ml of the sample diluent into the control well (Zero well). Cover with an adhesive strip and incubate at 37 °C for 90 min.
   Note: We recommend that each Human ACE2 standard solution and each sample is measured in duplicate.
2. Aspirate each well, and blot the plate onto paper towels or other absorbent material. Do not wash and do NOT let the wells completely dry at any time.
3. Add 100 µl of the Detection Antibody working solution to each well. Cover with a new adhesive strip and incubate at 37 °C for 60 min.
4. Aspirate each well and wash with Wash Buffer, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or auto-washer. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
5. Add 100 µl of the working solution of Streptavidin-HRP to each well. Cover the plate and incubate at 37 °C for 30 min. Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 4 for three times.
7. Add 90 µl of TMB substrate solution to each well. Cover and incubate at room temperature for 20 -45 min until a gradient develops and you see visible color in the 2nd lowest concentration well. Protect from light. Do not over-develop.
8. Add 100 µl Stop Solution to each well. Mix well.
9. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader immediately.

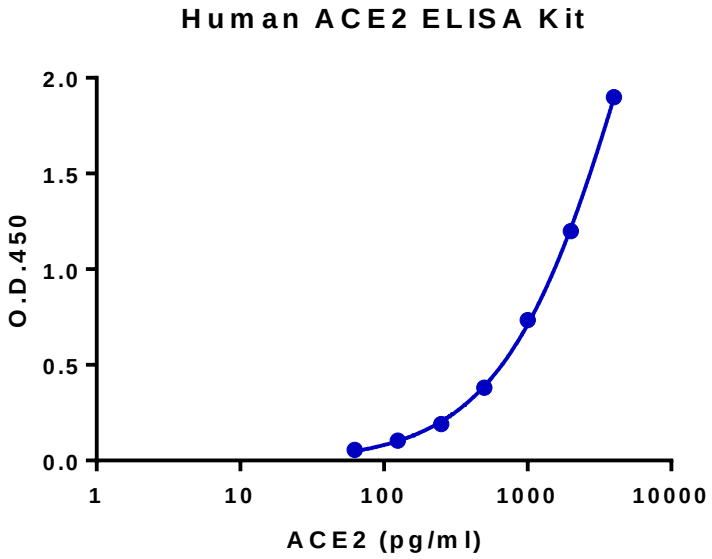
Result calculation

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Human ACE2 concentration of the samples can be interpolated from the standard curve.

Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution

Typical data

This standard curve was generated at the Novatein Biosciences laboratory for demonstration purpose only. A standard curve must be run with each assay.



Sensitivity

The sensitivity or minimum detectable dose (MDD) of Human ACE2 was determined to be 12.5 pg/ml. MDD is defined as the human ACE2 concentration resulting in an $O.D._{450}$ value that is 2 standard deviations higher than blank.

Spiking and Recovery

Recovery was determined by spiking the following matrices with various concentrations of human ACE2.

Sample Type	Average Recovery (%)	Range (%)
Cell Culture Supernatant	91.1	84-97
Serum	98.3	89-101

Reproducibility

- Inter-assay- <9.4%
- Intra-assay- <7.7%

Specificity

This kit recognizes both natural and recombinant human ACE2.

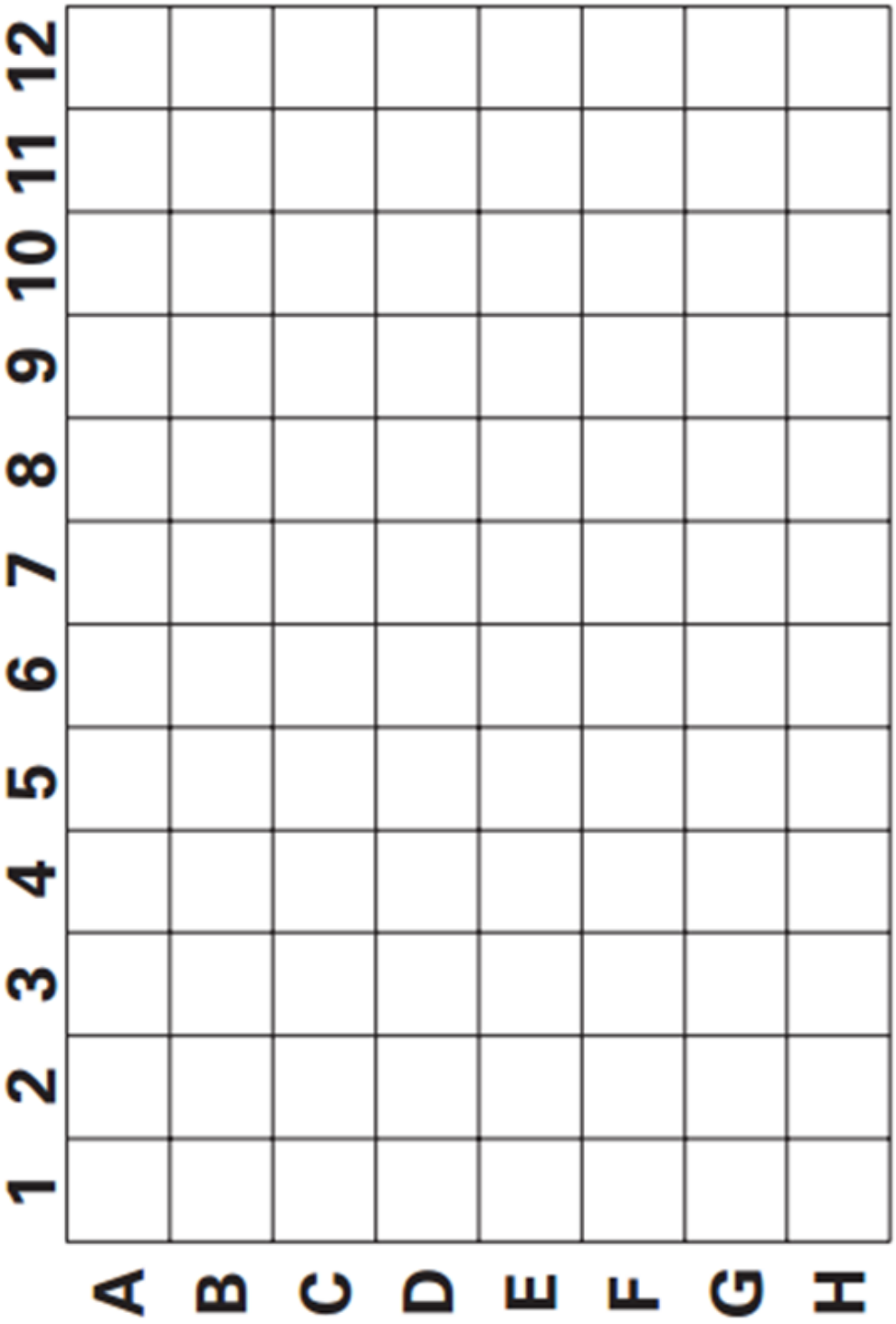
Sample Dilution

The user may need to determine the dilution factor in a preliminary experiment. If required, samples should be diluted in sample diluent buffer.

For trouble shooting information please visit the following website:
http://www.novateinbio.com/en/content/15-tech-info OR

Email us at techsupport@novateinbio.com

Plate Layout



Troubleshooting Information

High Background

Probable Cause:	Solution/ Action
High incubation temperature:	Incubate at room temperature (25 °C) throughout the procedure
Insufficient washing of the plate:	Fill the wells with wash buffer and aspirate completely for the next wash Increase the number of washes Add soak time (20-30 seconds) in between the washes Use automated plate washer, if available and check that all the channels are operating properly
Concentrated streptavidin-HRP	Streptavidin-HRP was not diluted properly Dilute the streptavidin-HRP as mentioned in the manual
Light exposure during substrate incubation	The TMB substrate is light sensitive and turns to blue color in the presence of light. The incubation must be carried out in dark.
Stop solution not added	Color will continue to develop if stop solution is not added
Diluents came with the kit were not used	Standards/ sample, detection antibody and streptavidin-HRP must be diluted in the respective buffers came with the kit. Do not use buffers from other kits
Contaminated solutions	Prepare fresh working solutions

Poor Standard Curve

Probable Cause:	Solution/ Action
Improper standard reconstitution:	Spin the vial briefly before opening Reconstitute the standard as mentioned in the manual. After reconstitution, leave it atleast for 10 minutes at room temperature Do not store and reuse diluted standards
Curve fitting problem:	Log transform the values on both axes Use 4-PL/ 5-PL curve fitting programs
Incubation temperature/ time	Use the recommended standard incubation conditions
Poor dilutions	Pipetting error. Check pipetting technique and calculations. Use calibrated pipettes.

No Signal

Probable Cause:	Solution/ Action
Omission of reagent(s):	Read the manual entirely. Check that all the reagents are added in the correct order as stated in the manual
Incorrect detection antibody was used:	Use the detection antibody came with the kit
Chromogen solutions were mixed improperly	Use the recommended procedure to prepare the TMB substrate
HRP inhibitor in sample/ buffers	Check that the samples/ buffers do not have sodium azide as it will inhibit peroxidase reaction.
Vigorous washing	If the washing is done manually, pipette the wash buffer gently.
Dried wells	Do not allow the wells to dry out during the assay. Seal with the supplied adhesive cover during incubations
Improper plate reader settings	Check the wavelength and read the plate again

Erratic duplicate OD values

Probable Cause:	Solution/ Action
Insufficient washing of the plate	Fill the wells with wash buffer and aspirate completely for the next wash Increase number of washes Add soak time (20-30 seconds) in between the washes Use automated plate washer, is available and check that all the channels are functioning properly
Poor dilutions	Pipetting error. Check pipetting technique and calculations. Use calibrated pipettes.
Improper mixing of samples/ buffers	Mix the samples well before pipetting Thoroughly mix the working solutions of detection antibody/ streptavidin-HRP
Contamination from other wells	Do not reuse the adhesive covers from previous assay setups Change pipette tips during reagent addition. If same pipette tip is being used to dispense reagents, care should be taken, not to touch the solution in the well
Precipitates in the samples/ buffer	If precipitates are visible in wash buffer concentrate, keep it at 37 ^{o}C for 10-15 minutes until no precipitates are visible Centrifuge the samples to remove particulate matter
Dried wells	Do not allow the wells to dry out during the assay. Seal with the supplied adhesive cover during incubations