ACE2 ELISA Kit

(For Rat)

Enzyme Immunoassay for the quantification of rat Angiotensin I Converting Enzyme 2 (ACE2) in serum, plasma and cell culture supernatant

Catalog number: ARG80968

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INTRODUCTION

Carboxypeptidase which converts angiotensin I to angiotensin 1-9, a peptide of unknown function, and angiotensin II to angiotensin 1-7, a vasodilator. Also able to hydrolyze apelin-13 and dynorphin-13 with high efficiency. May be an important regulator of heart function. In case of human coronaviruses SARS and HCoV-NL63 infections, serve as functional receptor for the spike glycoprotein of both coronaviruses. [UniProt]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for rat ACE2 has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any ACE2 present is bound by the immobilized antibody. After washing away any unbound substances, a HRP-conjugated antibody specific for ACE2 is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a chromogenic 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 by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of ACE2 in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	96 wells	4°C.
Standards A-F (Concentration: 1.56, 3.12, 6.25, 12.5, 25, 50 U/L)	6 vials X 0.5ml	4°C
Sample Diluent	6 ml	4°C
HRP-conjugated Antibody	10 ml	4°C
20X Wash buffer	25 ml	4°C
Chromogen solution A	6 ml	4°C (Protect from light)
Chromogen solution B	6 ml	4°C (Protect from light)
Plate sealer	2	RT
STOP solution	6 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- 37 °C incubator
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatants - Remove particulates by centrifugation and aliquot & store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

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 or heparin 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.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer.
- Samples: If samples give OD readings higher than the highest standard provided, please dilute samples with sample diluent and repeat the assay.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards and samples should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 50 µl Sample Diluent Buffer in duplicate into wells (blank).
3. Add 50 µl of standards and samples in duplicate into wells.
4. Add 100ul of HRP-conjugated antibody to each well.
5. Mix well gently and incubate the plate for 1 hour at 37°C.
6. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1× Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
7. Add 50 µl of Chromogen solution A and 50 µl of Chromogen solution B to each well subsequently. Cover wells and incubate for 15 minutes at 37°C.
8. Add 50 µl of Stop Solution to each well. Mix well.
9. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards and samples.

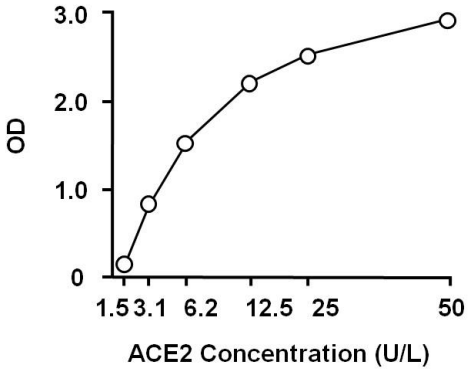
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of ACE2 ranged from 1.56-50 U/L. The mean MDD was 0.1 U/L.