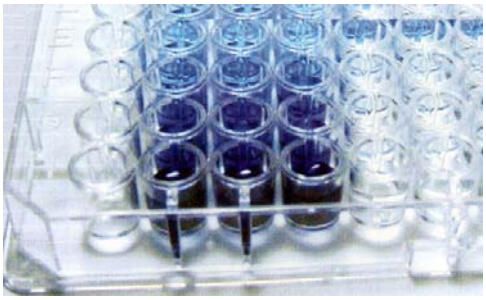
# Human ACE2 ELISA Kit

**Cat. No. AE0631TP**



***Instruction Manual***
**Version 1.0.0**

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

AdipoGen
Connecting Immunology to Metabolism

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## Introduction

By EST database searching for sequences showing homology to the zinc metalloprotease angiotensin-I converting enzyme, a full-length ACE2 cDNA, originally called ACEH, was isolated, which encoded a deduced 805-amino acid protein that shares approximately 40% identity with the N- and C-terminal domains of ACE. ACE2 contains a potential 17-amino acid N-terminal signal peptide and a putative 22-amino acid C-terminal membrane anchor. Northern blot analysis detected high expression of ACE2 in kidney, testis, and heart, and moderate expression in colon, small intestine, and ovary. Tipnis et al.(1) expressed a soluble, truncated form of ACE2 lacking transmembrane and cytosolic domains in CHO cells and found that it produced a glycosylated protein that was able to cleave angiotensin I and angiotensin II , but not bradykinin. Boehm and Nabel (2) showed whereas ACE converts angiotensin I to angiotensin II, which has 8 amino acids, ACE2 converts angiotensin I to angiotensin 1-9, which has 9 amino acids. Which can then further be converted by ACE to a shorter peptide, angiotensin 1-7, which is a blood vessel dilator. Using ACE2 null mice, Crackower et al. showed that ACE2 was critically involved in a cardiac contractility (3). Li et al.(4) identified ACE2, isolated from SARS coronavirus-permissive Vero E6 cells, that efficiently binds the S1 domain of the SARS coronavirus S protein. It was shown that when ACE2 was engaged with S protein ACE2 surface expression was down-regulated (5) , increasing lung levels of angiotensin II, which was proposed to give rise to a severe lung injury. Since ACE2 is detected in urine (1), measurement of shedded ACE2 may provide a novel clue to renal & cardiovascular function of ACE2.

## Assay Principles

This kit is an enzyme-linked immunosorbent assay (ELISA) for quantitative determination of ACE2 in human urine samples or cell culture supernatants.

Polyclonal antibody specific for human ACE2 has been pre-coated onto 96 well microplate. Standards and samples are pipetted into the wells and any ACE2 present is bound by immobilized antibody. Bound ACE2 is captured by biotinylated anti-human ACE2 polyclonal antibody. HRP conjugated streptavidin is added. After washing, a substrate solution is added. The colors develop in proportion to the bounded ACE2 quantity. The color development is stopped and the intensity of color is measured.

## Kit Components

1) Antibody coated 96-well plate, 12X 8-well strips
2) 5X Wash concentrate, 100 ml
3) 5X Diluent, 50 ml
4) Secondary antibody, 12 ml
5) 100X Detector, 150µl
6) Standard, recombinant human ACE2 expressed by HEK 293 cells, 1 vial, lyophilized
7) QC sample = a positive control of recombinant human ACE2 protein, 1 vial, lyophilized (For actual concentration of QC sample, see the 'Certificate of analysis' enclosed)
8) Substrate solution, 12ml
9) Stop solution, 12 ml
10) plate sealers, 3 sealers

## Reagents Description

**Antibody coated 96-well plates**, 12X 8-well strips, with absorbed polyclonal antibody against human ACE2
**5X Wash concentrate**, buffered detergent solution, supplied as a 5X concentrate
**5X Diluent**, for sample and reagent dilution
**1X Secondary antibody**, biotinylated polyclonal antibody against human ACE2
**100X detector**, HRP conjugated streptavidin
**Substrate solution**, chromogenic reagents
**Stop solution**, 1M $H_3PO_4$
**Standard, 50.0 ng**, recombinant human ACE2

## Storage of Reagents

Reagents must be stored at 2-8°C when not in use. Reagents must be brought to room temperature before use. Do not expose reagents to temperature greater than 25°C. Diluted wash solution may be stored at room temperature for up to one month.

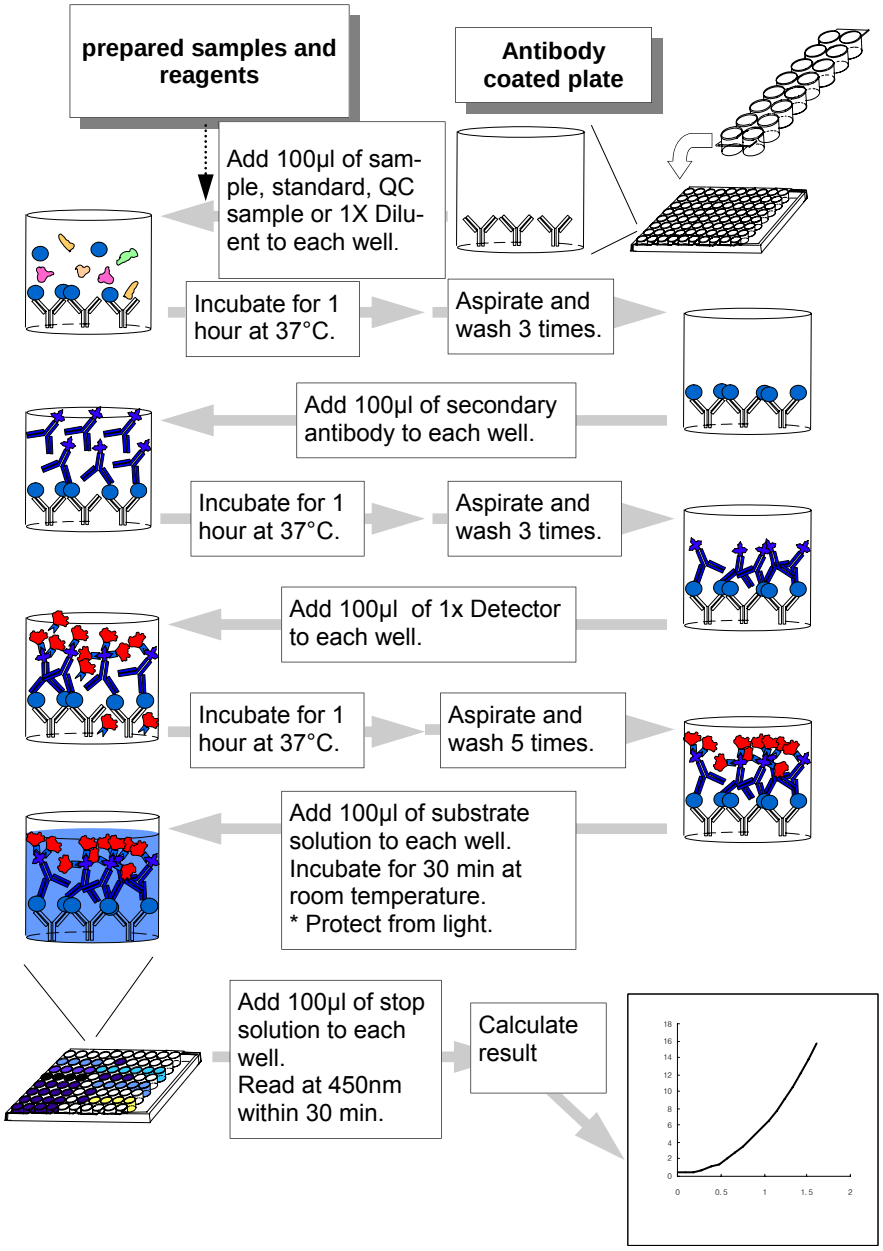
## Materials Required but not Supplied

Precision single and multi-channel pipettes.
Disposable pipette tips.
Microtubes or equivalent for preparing dilutions.
Disposable plastic containers for preparing working reagents.
Reagent reservoirs.
Microwell or microstrip plate reader 450 nm
Deionized water

## Sample Collection and Storage

**Urine** Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter. Assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

## Flow Chart of Assay Procedure

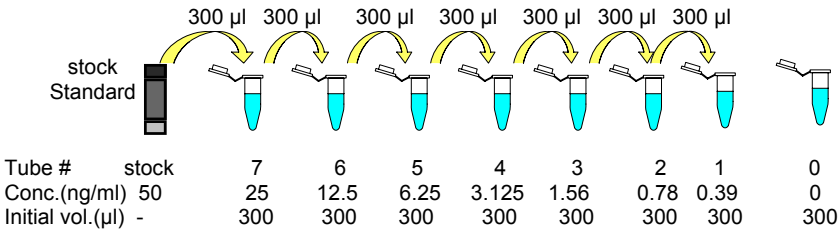


## Assay Procedure

**1. Preparation of Reagents**

1) Allow all samples and kit components to equilibrate to room temperature (20-25°C).
2) Plan the plate configuration and create a plate map. Calculate the amount of working reagents to use.
   It is recommended that Standards and samples be run in duplicate.
3) Prepare **1X Wash Solution**. Dilute 5X Wash Concentrate 1:5 with deionized water (1 part 5X Wash Concentrate with 4 parts deionized water). The diluted 1X Wash Solution is stable for one month at room temperature.
4) Prepare **1X Diluent**. Dilute 5X Diluent 1:5 with deionized water (1 part 5X Diluent with 4 parts deionized water).
5) Prepare **1X Detector**. Dilute 100X Detector 1:100 with 1X Diluent (1 part 100X Detector with 99 parts 1X Diluent). Use the 1X Detector within one hour of preparation.
6) Warm **Substrate Solution** to room temperature before use.
7) Prepare working aliquots of the Standard as follows :
   When opening the lyophilized Standard, remove cap gently as the lyophilizate may have become dislodged during shipping. Add 1 ml of deionized water to the Standard vial to make a stock concentration of 50 ng/ml. Mix well. A recommended dilution scheme is as follows :

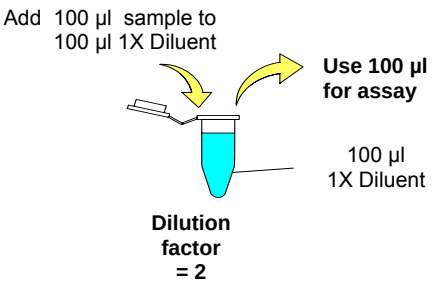
a) Label 8 microcentrifuge tubes #0-7. Add 300 µl of the 1X Diluent to the microcentrifuge tubes #0-7.
b) Add 300 µl of the stock Standard solution to tube #7 and vortex. This is Standard tube #7 with a concentration of 25 ng/ml.
c) Standards #6 to #1 are then prepared by performing a 1:2 dilution of the preceding Standard. Do not add any Standard to the tube #0.




8. Reconstitute QC sample in 1 ml of deionized water.

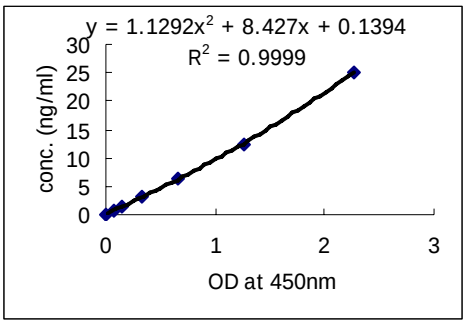
**2. Sample preparation**

1) Dilute urine samples 1:2 with 1X Diluent (for example,
100 µl sample plus 100 µl 1X Diluent, final 1:2).
2) Use 100 ul of the final diluted sample for ELISA.
   * If samples fall the outside range of assay, a lower or higher dilution may be required.



**3. Experiment procedure**

1) Remove the appropriate number of microwell strips from the sealed foil pouch.
2) Pipette 100 µl of Standards #0 to #7, the reconstituted QC sample and pre-treated sample into the antibody-coated plate according to the plate configuration. Use a new pipette tip for each Standard or sample.
3) Incubate at 37°C for 1 hour.
4) Remove the solution and wash 3 times with 300 µl of 1X Wash Solution per well.
5) Add 100 µl Secondary Antibody to each well.
6) Incubate at 37°C for 1 hour.
7) Remove the solution and wash 3 times with 300 µl of 1X Wash Solution per well.
8) Add 100µl 1X Detector to each well.
9) Incubate at 37°C for 1 hour.
10) Remove the solution and wash 5 times with 350 µl of 1X Wash Solution per well.
11) Using the multi-channel pipette, add 100 µl of the room temperature Substrate Solution to each well.
12) Incubate at room temperature for 30 min.
    * Protect from light.
13) Using the multi-channel pipette, add 100 µl Stop Solution to each well.
14) Read at 450 nm.
15) Subtract the absorbance of the blank from the readings for each Standard and sample.




16) Construct a Standard curve by plotting the known concentrations (Y) of Standard versus the absorbances (X) of Standard. A measurable range is typically shown between 0.39 ng/ml and 25 ng/ml.
17) Calculate the ACE2 concentrations of samples by interpolation of the regression curve formula as shown above in a form of a quadratic equation.
18) The ACE2 concentrations calculated must be multiplied by dilution factor to obtain the concentrations of the undiluted samples (Dilution factor of lyophilized QC sample is 2).

## Performance Characteristics

**1) Sensitivity :** 293 pg/ml

**2) Precision**

**a. Intra-Assay (precision within an assay)**

6 urine samples were tested 11 times to assess intra-assay precision.

| Sample | Mean (ng/ml) | SD (ng/ml) | CV(%) |
|---|---|---|---|
| 1 | 2.785 | 0.169 | 6.079 |
| 2 | 4.120 | 0.254 | 6.164 |
| 3 | 12.680 | 1.257 | 9.914 |
| 4 | 15.034 | 0.807 | 5.365 |
| 5 | 24.705 | 1.319 | 5.338 |
| 6 | 45.697 | 3.724 | 8.149 |

**b. Inter-Assay (precision between assays)**

6 urine samples were tested 11 times to assess inter-assay precision.

| Sample | Mean (ng/ml) | SD (ng/ml) | CV(%) |
|---|---|---|---|
| 1 | 4.105 | 0.445 | 10.829 |
| 2 | 8.726 | 0.617 | 7.069 |
| 3 | 11.280 | 1.121 | 9.937 |
| 4 | 16.913 | 1.045 | 6.179 |
| 5 | 22.861 | 1.235 | 5.401 |
| 6 | 49.219 | 3.144 | 6.388 |

### 3) Recovery

The recovery of ACE2 spiked to three different levels in five different urine samples throughout the range of assay was evaluated.

| Sample No. | Average recovery (%) | Range (%) |
|---|---|---|
| 1 | 90.8 | 81~97 |
| 2 | 93.4 | 84~109 |
| 3 | 95.1 | 84~111 |
| 4 | 95.4 | 89~105 |
| 5 | 100.7 | 90~113 |

### 4) Linearity - Effect of Urine Dilution

Three urine samples were pre-treated as described in the protocol, resulting in the final dilution of x2(labeled in the table below as dilutin;1)

| Sample No. | Serum Dilution | Expected (ng/ml) | Observed (ng/ml) | % Of Expected |
|---|---|---|---|---|
| 1 | 1 | 5.928 | 5.928 | 100.0 |
| | 1/2 | 2.964 | 3.027 | 102.1 |
| | 1/4 | 1.482 | 1.403 | 94.7 |
| 2 | 1 | 8.868 | 8.868 | 100.0 |
| | 1/2 | 4.434 | 4.724 | 106.5 |
| | 1/4 | 2.217 | 2.116 | 95.4 |
| 3 | 1 | 19.314 | 19.314 | 100.0 |
| | 1/2 | 9.657 | 10.014 | 103.7 |
| | 1/4 | 4.829 | 4.315 | 89.4 |

**5) Specificity -** Cross Reactivity of recombinant proteins

| Analyte | Max. Conc. (ng/ml) | Cross Reactivity (%) |
|---|---|---|
| Human ACE2 | 10 | 100 |
| Human Adiponectin | 100 | N. R. |
| Human leptin | 100 | N. R. |
| Human resistin | 100 | N. R. |
| Human visfatin | 100 | N. R. |
| Human clusterin | 100 | N. R. |
| Human RBP4 | 100 | N. R. |
| Human RELMbeta | 100 | N. R. |
| Mouse RELMalpha | 100 | N. R. |
| Human IL23 | 100 | N. R. |
| Human ANG1 | 100 | N. R. |
| Human ANG2 | 100 | N. R. |
| Human FABP4 | 100 | N. R. |
| Human AGF | 100 | N. R. |
| Human PAI-1 | 100 | N. R. |
| Human vaspin | 100 | N. R. |

**N. R. : No Cross-reactivity**

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| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | | | | | | | | | | | | |
| B | | | | | | | | | | | | |
| C | | | | | | | | | | | | |
| D | | | | | | | | | | | | |
| E | | | | | | | | | | | | |
| F | | | | | | | | | | | | |
| G | | | | | | | | | | | | |
| H | | | | | | | | | | | | |

## Troubleshooting Guide

| Problem | Possible Cause | Solution |
|---|---|---|
| No signal or weak signal | Omission of key reagent | Check that all reagents have been added in the correct order. |
| | Washes too stringent | Use an automated plate washer if possible. |
| | Incubation times inadequate | Incubation times should be appropriate for the system. |
| | Plate reader settings not optimal | Verify the wavelength and filter setting in the plate reader. |
| | Incorrect assay temperature | Use recommended incubation temperature. Bring substrates to room temperature before use. |
| High background | Concentration of detector too high | Use recommended dilution factor. |
| | Inadequate washing | Ensure all wells are filling wash buffer and are aspirated completely. |
| Poor standard curve | Wells not completely aspirated | Completely aspirate wells between steps. |
| | Reagents poorly mixed | Be sure that reagents are thoroughly mixed. |
| Unexpected results | Omission of reagents | Be sure that reagents were prepared correctly and added in the correct order. |
| | Dilution error | Check pipetting technique and double-check calculations. |
| | Technique problem | Proper mixing of reagents and wash steps are critical. |

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