APB886Hu61 100μg
Active Angiotensin I Converting Enzyme 2 (ACE2)
Organism Species: *Homo sapiens (Human)*
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Prokaryotic expression.
Host: *293F cell*
Residues: Gln18~Ser740
Tags: N-terminal His-tag
Purity: >95%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: PBS, pH7.4, containing 5% Trehalose .
Original Concentration: 200μg/mL
Applications: Cell culture; Activity Assays.
(May be suitable for use in other assays to be determined by the end user.)
Predicted isoelectric point: 5.1
Predicted Molecular Mass: 85.2kDa
Accurate Molecular Mass: 80kDa as determined by SDS-PAGE reducing conditions.

[USAGE]

Reconstitute in 10mM PBS (pH7.4) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.
Store at 2-8ºC for one month.
Aliquot and store at -80ºC for 12 months.

Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[SEQUENCE]

QST IEEQAKTFLD KFNHEAEDLF YQSSLASWNY
NTNITEENVQ NMNNAGDKWS AFLKEQSTLA QMYPLQEIQN LTVKLQLQAL
QQNGSSVLSE DKSKRLNTIL NTMSTIYSTG KVCNPDNPQE CLLLEPGLNE
IMANSLDYNE RLWAWESWRS EVGKQLRPLY EEYVVLKNEM ARANHYEDYG
DYWRGDYEVN GVDGYDYSRG QLIEDVEHTF EEIKPLYEHL HAYVRAKLMN
AYPSYISPIG CLPAHLLGDM WGRFWTNLYS LTVPFGQKPN IDVTDAMVDQ
AWDAQRIFKE AEKFFVSVGL PNMTQGFWEN SMLTDPGNVQ KAVCHPTAWD
LGKGDFRILM CTKVTMDDFL TAHHEMGHIQ YDMAYAAQPF LLRNGANEGF
HEAVGEIMSL SAATPKHLKS IGLLSPDFQE DNETEINFLL KQALTIVGTL
PFTYMLEKWR WMVFKGEIPK DQWMKKWWEM KREIVGVVEP VPHDETYCDP
ASLFHVSNDY SFIRYYTRTL YQFQFQEALC QAAKHEGPLH KCDISNSTEA
GQKLFNMLRL GKSEPWTLAL ENVVGAKNMN VRPLLNYFEP LFTWLKDQNK
NSFVGWSTDW SPYADQSIKV RISLKSALGD KAYEWNDNEM YLFRSSVAYA
MRQYFLKVKN QMILFGEEDV RVANLKPRIS FNFFVTAPKN VSDIIPRTEV
EKAIRMSRSR INDAFRLNDN SLEFLGIQPT LGPPNQPPVS

[ACTIVITY]

Angiotensin I Converting Enzyme 2 (ACE2), as a transmembrane protein, serves as the main entry point into cells for some coronaviruses. More specifically, the binding of the spike S1 protein of SARS-CoV and SARS-CoV-2 to the enzymatic domain of ACE2 on the surface of cells results in endocytosis and translocation of both the virusand the enzyme into endosomes located within cells. Besides, recent studies show that spike (S) proteins of 2019-nCoV and SARS-CoV may use the same host cell receptor called angiotensin-converting enzyme 2 (ACE2) for entering into host cells, thus a binding ELISA assay was conducted to detect the interaction of recombinant human ACE2 and recombinant Spike glycoprotein RBD.

Briefly, biotin-linked ACE2 were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100 µl were then transferred to RBD-coated microtiter wells and incubated for 2h at 37℃. Wells were washed with PBST 3 times and incubation with HRP conjugage for 30min, then wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37℃. Finally, add 50 µl stop solution to the wells and read at 450 nm immediately. The binding activity of ACE2 and RBD was shown in Figure 1, and this effect was in a dose dependent manner.

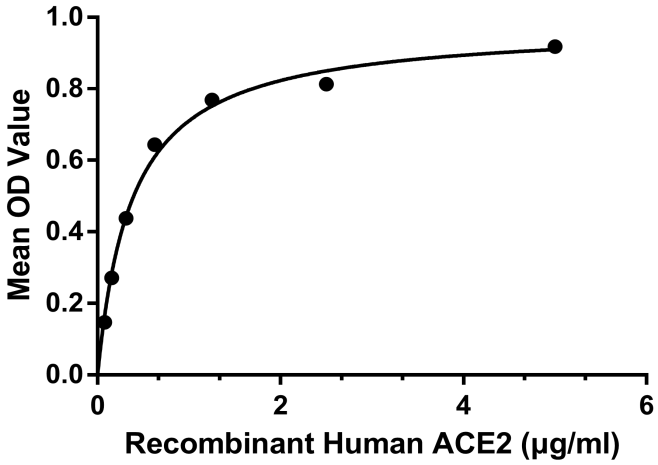
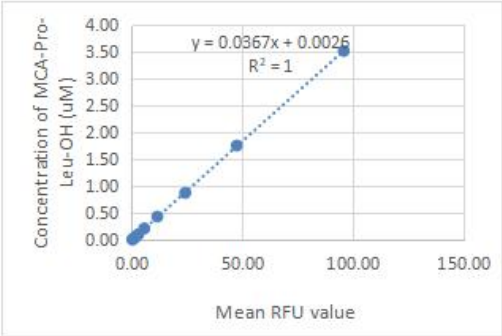


Figure 1. The binding activity of ACE2 with RBD

The activity of recombinant human ACE2 is also measured by its ability to cleave a fluorogenic peptide substrate MCA-Tyr-Val-Ala-Asp-Ala-Pro-Lys(DNP)-OH in the assay buffer 50 mM Tris, 1 M NaCl, pH 7.5. The rhACE2 is diluted to 0.5 ug/mL in assay buffer. Loading into a black well plate 50 µL of 0.5 ug/mL rhACE2 and start the reaction by adding 50 µL of 20 µM substrate, with a substrate blank containing 50 µL assay buffer, 50 µL substrate, and no rhACE2. Then read at excitiation and emission wavelengths of 320 nm and 405 nm, respectively, in kinetic mode for 5 minutes. The specific activity of recombinant human ACE2 is > 1100 pmol/min/µg.



RFU (320/405)	MCA-Pro-Leu-OH (product) uM
95.78	3.52
47.46	1.76
24.20	0.88
11.63	0.44
5.71	0.22
3.05	0.11
1.52	0.05
0.77	0.03

Figure 2. The standard curve of MCA-Pro-Leu-OH

Specific Activity (pmol/min/μg) =

$$\frac{\text{Adjusted Vmax}*\text{(RFU/min)} \times \text{Conversion Factor}**\text{(pmol/RFU)}}{\text{amount of enzyme (ug)}}$$

*Adjusted for Substrate Blank

**Derived using calibration standard MCA-Pro-Leu-OH

[IDENTIFICATION]

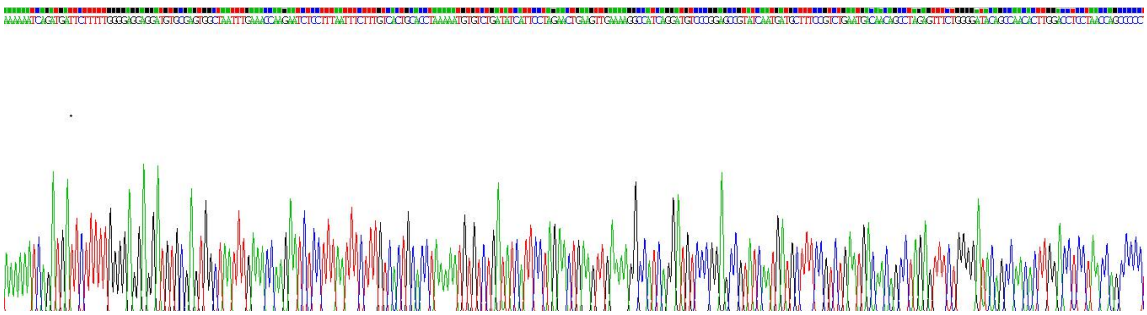


Figure 3. Gene Sequencing (extract)

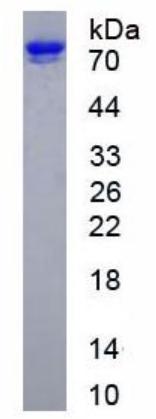


Figure 4. SDS-PAGE

Sample: Active recombinant ACE2, Human

[IMPORTANT NOTE]

The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.