Recombinant Human ACE2-Fc Chimera (carrier-free)

Catalog# / Size	793202 / 10 µg 793204 / 25 µg 793206 / 100 µg 793208 / 500 µg
Regulatory Status	RUO
Other Names	Angiotensin I Converting Enzyme 2, Angiotensin I Converting Enzyme (Peptidyl-Dipeptidase A) 2, Angiotensin-Converting Enzyme Homolog, ACE-Related Carboxypeptidase, Peptidyl-Dipeptidase A, ACEH, ACE-2
Description	ACE2 is a member of the angiotensin-converting enzyme family of dipeptidyl carboxydipeptidases. It catalyzes the cleavage of the decapeptide angiotensin I into angiotensin-(1-9) and angiotensin II (potent vasoconstrictor) into the vasodilator angiotensin-(1-7). ACE2 is a type I membrane protein that functions as a carboxypeptidase. It cleaves between a proline and a single hydrophobic/basic residue from the COOH-terminus of its substrates. The human full-length enzyme possesses 805 amino acids and the ECD includes amino acids 1–740. ACE2 is a zinc metalloprotease with considerable homology to angiotensin I-converting enzyme (ACE), both enzymes contain the typical HEXXH zinc-binding motif, ACE has two catalytic sites and ACE2 has only one, and ACE2 is not inhibited by ACE inhibitors captopril, lisinopril, and enalaprilat. Studies in mice showed that disruption of ACE2 induced a severe cardiac contractility defect and increased angiotensin II levels in heart. Human ACE2 has been identified as the receptor for SARS (severe acute respiratory syndrome)-coronavirus. ACE2 binds to the coronavirus S protein present on the surface of the virion. The S protein is a type I protein with four domains that include an S1 (receptor binding subunit), an S2 (membrane-fusion subunit), a transmembrane, and a short intracellular domain. The S protein forms a trimer showing a big protrusion from the virus surface. Studies in SARS-CoV-ACE2 interactions showed that specific amino acids (Lys31 and Lys353) in human ACE2 were critical to virus-receptor binding, and naturally selected viral mutations in S1 (K479N and S487T) enhanced the affinity of S1 for human ACE2.

Product Details

Source	Human ACE2, amino acid (Gln18 - Ser740) (Accession: NM_021804.1) was expressed in CHO cells. The C-terminus contains SR-IEGRMD- hIgG1(Pro100-Lys330)-Fc tag.
Molecular Mass	The 962 amino acid recombinant protein has a predicted molecular mass of approximately 110.48 kD. The DTT-reduced and non reduced protein migrates at approximately 130 kD and 250 kD respectively by SDS-PAGE. The C-terminus contains SR-IEGRMD- hIgG1(Pro100-Lys330)-Fc tag. The predicted N-terminal amino acid is Gln.
Purity	> 95%, as determined by Coomassie stained SDS-PAGE
Formulation	0.22 µm filtered protein solution is in 50 mM Tris, 200 mM NaCl, 50 µM $ZnCl_2$, and 5% glycerol, pH 7
Endotoxin Level	Less than 0.1 EU per µg cytokine as determined by the LAL method
Concentration	10 and 25 µg sizes are bottled at 200 µg/mL. 100 µg and larger sizes are lot-specific and bottled at the concentration indicated on the vial. To obtain lot-specific concentration, please enter the lot number in our Concentration and Expiration Lookup or Certificate of Analysis online tools.
Storage & Handling	Unopened vial can be stored at -20°C or -70°C for six months. For maximum results, quick spin vial prior to opening. **Avoid repeated freeze/thaw cycles**.
Activity	Human angiotensin I-converting enzyme 2 (ACE2) cleaves the fluorogenic peptide substrate, Mca-YVADAPK(Dnp)-OH. The specific activity is > 300 pmol/min/µg in the presence of 0.013 µg of recombinant human ACE2. When recombinant SARS-CoV-2 S protein S1 is immobilized at 2 µg/mL, recombinant human ACE2-Fc chimera binds in a dose-dependent manner. The ED50 range for this effect is 10 - 50 ng/mL. HRP Protein A (Cat. No. 689202) was used to detect the binding.
Application	Bioassay
Application Notes	Human ACE2 Enzymatic Assay

Human angiotensin I-converting enzyme 2 (ACE2) cleaves the fluorogenic peptide substrate, Mca-YVADAPK(Dnp)-OH. The increase of the product is monitored by an increase in intensity of fluorescence at 405 nm with excitation at 320 nm. The specific activity is > 300 pmol/min/µg in the presence of 0.013 µg of recombinant human ACE2.

Materials

- Assay buffer: 75 mM Tris, 1 M NaCl, pH 7.5
- Recombinant human ACE2
- Substrate: MCA-Tyr-Val-Ala-Asp-Ala-Pro-Lys(DNP)-OH, 10 mM stock in DMSO (Enzo, Cat. No. ALX-260-023-M001)
- F16 Black Maxisorp plate (Nunc, Cat. No. 475515)
- Fluorescent plate reader (Model: SpectraMax M3 by Molecular Devices) or equivalent

Procedure

1. Dilute the recombinant human ACE2 to 0.26 ng/µL in assay buffer.
2. Dilute the substrate to 40 µM in assay buffer.
3. Load in a black well plate 50 µL of 0.2 ng/µL of recombinant human ACE2, and start the reaction by adding 50 µL of 40 µM substrate. As a control load 50 µL of 40 µM substrate with 50 µL of assay buffer.
4. Read at excitation and emission wavelengths of 320 nm and 405 nm (top read), respectively in kinetic mode for 5 minutes.
5. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted Vmax* (RFU/min) x Conversion Factor** (pmol/RFU)}}{\text{amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank
**Derived using calibration standard MCA-Pro-Leu-OH (Bachem, Cat. No. M-1975)

Per Well:
Recombinant human ACE2: 0.013 µg
Substrate: 20 µM

BioLegend carrier-free recombinant proteins provided in liquid format are shipped on blue-ice. Our comparison testing data indicates that when handled and stored as recommended, the liquid format has equal or better stability and shelf-life compared to commercially available lyophilized proteins after reconstitution. Our liquid proteins are verified in-house to maintain activity after shipping on blue ice and are backed by our 100% satisfaction guarantee. If you have any concerns, contact us at tech@biolegend.com.

Product Citations

1. Kurup D, *et al.* 2020. NPJ Vaccines. 0.276388889. PubMed

Antigen Details

Structure	Dimer
Distribution	Vascular endothelial cells of the heart, kidney, brain, and testis
Function	Regulates renal and cardiovascular function
Interaction	Angiotensin I and angiotensin II are substrates for ACE2.
Ligand/Receptor	Spike glycoprotein expressed in human coronaviruses such as HCoV-NL63, SARS-CoV, and SARS-CoV-2.
Bioactivity	ACE2 cleaves the fluorogenic peptide substrate, Mca-YVADAPK(Dnp)-OH. The increase of the product is monitored by an increase in intensity of fluorescence at 405 nm with excitation at 320 nm.
Cell Type	Endothelial cells, Epithelial cells
Biology Area	Cardiovascular Biology, Cell Biology, COVID-19
Molecular Family	Enzymes and Regulators, Proteases

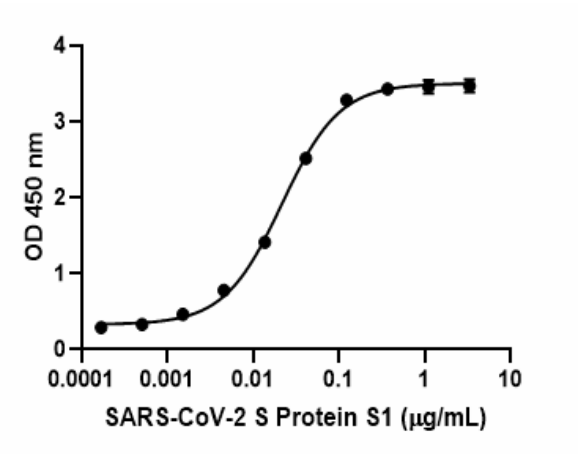
Antigen References

1. Crackower MA, *et al*. 2002. *Nature*. 417:822-8.
2. Vickers C, *et al*. 2002. *J Biol Chem*. 277:14838-43.
3. Tikellis C, *et al.* 2003. *Hypertension*. 41:392-7.
4. Guan Y, *et al*. 2003. *Science*. 302:276-8.
5. Li F, *et al*. 2005. *Science*. 309:1864-8.
6. Ferrario CM, *et al*. 2005. *Am J Physiol Heart Circ Physiol*. 289: H2281-90.
7. Perlman S & Netland J. 2009. *Nat Rev Microbiol*. 7:439-50.

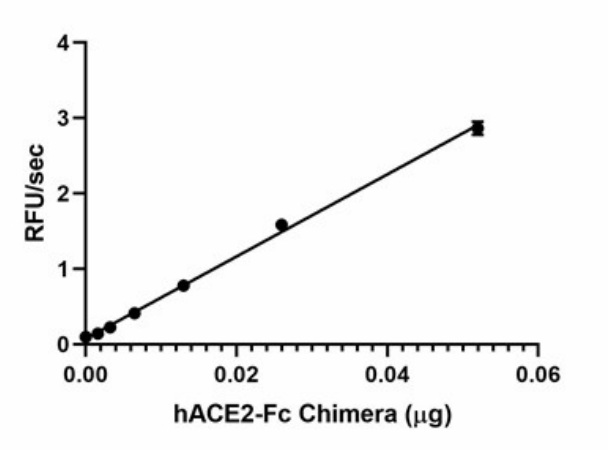
8. Wu K, *et al*. 2011. *J Virol*. 85:5331-7.
9. Ge XY, *et al*. 2013. *Nature*. 503:535-8.
10. Letko M, *et al.* 2020. *Nat Microbiol*. 5:562-569.

Gene ID 59272

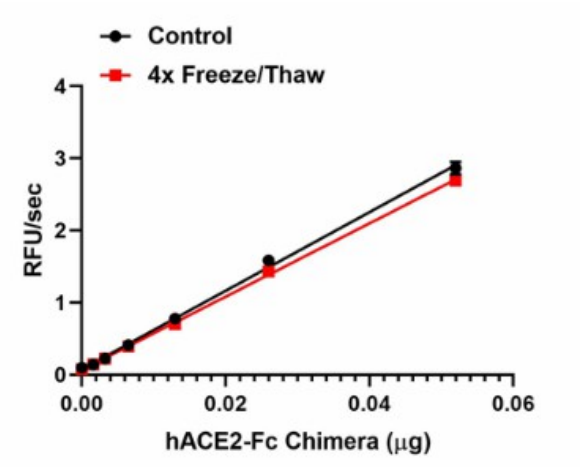
Product Data



Human ACE2-Fc Chimera binding assay with SARS-CoV-2 S Protein S1. When recombinant SARS-CoV-2 S Protein S1 is immobilized at 2 µg/mL, recombinant human ACE2-Fc Chimera binds in a dose-dependent manner. The ED_{50} range for this effect is 10 - 50 ng/mL. HRP Protein A (Cat. No. 689202) was used to detect the binding.



Recombinant human ACE2-Fc chimera activity is measured by its ability to hydrolyze the fluorogenic peptide substrate, Mca-YVADAPK(Dnp)-OH. The increase of the product was monitored by an increase in intensity of fluorescence at 405 nm with excitation at 320 nm. The specific activity is > 300 pmol/min/µg in the presence of 0.013 µg of recombinant human ACE2-Fc chimera.



Stability Testing for Recombinant Human ACE2. Recombinant human ACE2-Fc chimera was aliquoted in 50 mM Tris, 200 mM NaCl, 50 µM $ZnCl_2$, and 5% glycerol, pH 7. One aliquot was frozen and thawed four times (4x Freeze/Thaw) and compared to the control that was kept at 4°C (Control). The samples were tested for their ability to hydrolyze the fluorogenic peptide substrate, Mca-YVADAPK(Dnp)-OH. The increase of the product was monitored by an increase in intensity of fluorescence at 405 nm with excitation at 320 nm. The specific activity is > 300 pmol/min/µg in the presence of 0.013 µg of recombinant human ACE2-Fc chimera.

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