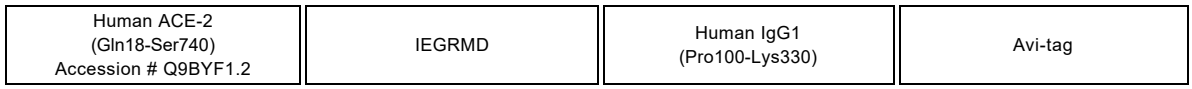
Biotinylated Recombinant Human ACE-2 Fc Chimera Avi-tag (CHO Expressed)

Catalog Number: AVI10544

DESCRIPTION

Source

Chinese Hamster Ovary cell line, CHO-derived human ACE-2 protein



N-terminal Sequence Analysis

Protein identity confirmed by mass spectrometry.

Structure / Form

Biotinylated via Avi-tag

Predicted Molecular Mass

112.0 kDa

SPECIFICATIONS

SDS-PAGE

118-130 kDa, under reducing conditions

Activity

Measured by its binding ability in a functional ELISA.
When Recombinant SARS-CoV-2 Spike RBD Fc Chimera (Catalog # 10499-CV) is immobilized at 0.2 µg/mL (100 µL/well), Biotinylated Recombinant Human ACE-2 Fc Chimera Avi-tag (CHO Expressed) (Catalog # AVI10544) binds with an ED_{50} of 0.6-4.8.

Measured by its ability to cleave a fluorogenic peptide substrate, Mca-YVADAPK(Dnp)-OH (Catalog # ES007).
The specific activity is >500 pmol/min/µg, as measured under the described conditions.

Endotoxin Level

<1.0 EU per 1 µg of the protein by the LAL method.

Purity

>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.

Formulation

Supplied as a 0.2 µm filtered solution in Tris, NaCl, $ZnCl_2$ and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 75 mM Tris, 1 M NaCl, pH 7.5
- Recombinant Human ACE-2 Fc Chimera Avi-tag (rhACE-2/Fc/Avi) (Catalog # AVI10544)
- Substrate: Mca-Tyr-Val-Ala-Asp-Ala-Pro-Lys(Dnp)-OH (Catalog # ES007) , 2 mM stock in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhACE-2/Fc/Avi to 0.8 µg/mL in Assay Buffer.
2. Dilute Substrate to 60 µM in Assay Buffer.
3. Load into a plate 50 µL of 0.8 µg/mL rhACE-2/Fc/Avi, and start the reaction by adding 50 µL of 60 µM Substrate. Include a Substrate Blank containing 50 µL of Assay Buffer and 50 µL of 60 µM Substrate.
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:

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

*Adjusted for Substrate Blank
**Derived using calibration standard MCA-Pro-Leu-OH (Bachem, Catalog # M-1975)

Final Assay Conditions

Per Well:

- rhACE-2/Fc/Avi: 0.04 µg
- Substrate: 30 µM

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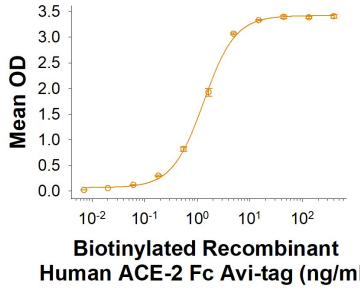
Catalog Number: AVI10544

PREPARATION AND STORAGE

Shipping	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 6 months from date of receipt, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after opening.

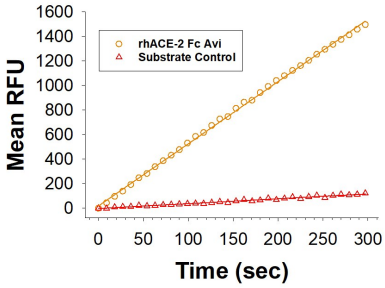
DATA

Binding Activity



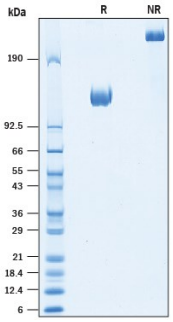
When Recombinant SARS-CoV-2 Spike RBD Fc Chimera (Catalog # 10499-CV) is immobilized at 0.2 µg/mL (100 µL/well), Biotinylated Recombinant Human ACE-2 Fc Chimera Avi-tag (CHO Expressed) (Catalog # AVI10544) binds with an ED_{50} of 0.6-4.8 ng/mL.

Enzyme Activity



Biotinylated Recombinant Human ACE-2 Fc Chimera Avi-tag (CHO Expressed) (Catalog # AVI10544) is measured by its ability to cleave a fluorogenic peptide substrate, Mca-YVADAPK(Dnp)-OH (Catalog # ES007).

SDS-PAGE



2 µg/lane of Biotinylated Recombinant Human ACE-2 Fc Chimera Avi-tag Protein (Catalog # AVI10544) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at ~124 kDa under reducing conditions.

BACKGROUND

Angiotensin I Converting Enzyme (ACE-2), also called ACEH (ACE homologue), is a dimeric, zinc-dependent metalloprotease of the ACE family that also includes somatic and germinal ACE (1, 2). ACE-2 mRNA is found at high levels in heart, testis, and kidney and at lower levels in a wide variety of tissues (1, 3). ACE-2 is the SARS-CoV and SARS-CoV2 Spike protein receptor *in vivo* (4-6), functions catalytically as a carboxypeptidase to cleave several substrates including angiotensins I and II, and acts as a partner for B0AT1-family amino acid transporters (1, 2). Through these functions, ACE-2 has been shown to be involved in several diseases including SARS, COVID19, acute lung injury (4, 7), heart disease (8), liver and lung fibrosis (9), inflammatory lung disease (10), and cardiopulmonary disease (11). Full length ACE-2 protein includes an extracellular region composed of a single N-terminal peptidase domain and C-terminal collectrin-like domain (CLD), a transmembrane domain, and a short cytoplasmic tail (12). The N-terminal peptidase region is required for binding to SARS-CoV and SARSCoV2 spike proteins, while the CLD contains a region that promotes dimerization and association with amino acid transporters (2). The peptidase domain contains a long deep cleft that undergoes a large hinge-bending movement at substrate and inhibitor binding (12). Classical ACE inhibitors such as captopril and lisinopril do not inhibit ACE-2 activity and inhibitors of ACE-2 do not inhibit ACE activity (13).

References:

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