Datasheet for ABIN6952755

COVID-19 Spike-ACE2 Binding Assay Kit

4 Images | 4 Publications

Go to Product page

Overview

Quantity:	96 tests
Target:	SARS-CoV-2 Spike, ACE2
Reactivity:	Human, SARS Coronavirus-2 (SARS-CoV-2)
Application:	Binding Studies (Bind), ELISA, Screening Assay (ScA)

Product Details

Purpose:	COVID-19 Spike-ACE2 binding assay kit is a rapid, simple, and sensitive method to characterize the binding affinity of the SARS-CoV-2 Spike (S) protein and the Angiotensin I Converting Enzyme 2 (ACE2) receptor complex in the presence of potential inhibitors.
Sample Type:	Plasma, Serum
Analytical Method:	Semi-Quantitative
Detection Method:	Colorimetric
Characteristics:	The COVID-19 Spike-ACE2 binding assay kit is a rapid, simple, and sensitive method to characterize the binding affinity of the SACE2 complex in the presence of potential inhibitors. The in vitro enzymelinked immunosorbent assay can measure numerous reagents and conditions simultaneously. For example, this kit can be used for screening inhibitor activity and drugs, vaccine development, and testing potential therapeutic antibodies.
Components:	1. COVID19 S-protein Microplate (Item A): 96 wells (12 strips x 8 wells) coated with recombinant COVID19 S-protein RBD domain. 2. Wash Buffer Concentrate (20x) (Item B): 25 ml of 20x concentrated solution. 3. Assay Diluent (Item E2): 15 ml of 5x concentrated buffer. For diluting testing reagent, ACE2 protein (Item F), detection antibody (Item C) and HRP-conjugated IgG Concentrate (Item D).

Product Details

	4. ACE2 protein (Item F): 2 vials of purified human recombinant ACE2 protein (1 vial is enough to assay half microplate) 5. Detection Antibody ACE2 (Item C-1): 2 vials of goat anti-ACE2 (1 vial is enough to assay half microplate). 6. HRP-conjugated Anti-goat IgG (Item D-1), 15 µl of 1000x concentrated HRP-conjugated anti-goat IgG. 7. TMB One-Step Substrate Reagent (Item H): 12 ml of 3,3',5,5'-tetramethylbenzidine (TMB) in buffered solution. 8. Stop Solution (Item I): 8 ml of 0.2 M sulfuric acid.
Material not included:	1. Microplate reader capable of measuring absorbance at 450 nm. 2. Shaker. 3. Precision pipettes to deliver 2 µL to 1 mL volumes. 4. Adjustable 1-25 mL pipettes for reagent preparation. 5. 100 mL and 1 liter graduated cylinders. 6. Distilled or deionized water. 7. Tubes to prepare sample dilutions.

Target Details

Target:	SARS-CoV-2 Spike, ACE2
Alternative Name:	SARS-CoV-2 Spike Protein & ACE2
Target Type:	Viral Protein

Application Details

Application Notes:	• Evaluate inhibitor activity • Screen inhibitors of the SARS-CoV-2 Spike-ACE2 complex • Characterize antibody function • Vaccine development • Selection of patient serum for antibody therapy
Sample Volume:	100 µL
Plate:	Pre-coated
Protocol:	The COVID-19 Spike-ACE2 binding assay uses a 96-well plate coated with recombinantly-expressed RBD of the SARS-CoV-2 Spike protein. The testing reagent-of-choice is then added to the wells in the presence of recombinant human ACE2 protein. Unbound ACE2 is removed with washing, and a goat anti-ACE2 antibody is added that binds to the Spike-ACE2 complex. HRP-conjugated anti-goat IgG is then applied to the wells in the presence of 3,3',5,5'-

Application Details

	tetramethylbenzidine (TMB) substrate. The HRP-conjugated anti-goat IgG binds to the ACE2 antibody and reacts with the TMB solution, producing a blue color that is proportional to the amount of bound ACE. The HRP-TMB reaction is halted with the addition of the Stop Solution, resulting in a blue-to-yellow color change. The intensity of the yellow color is then measured at 450 nm.
Sample Preparation:	Mix testing reagent (e.g., small molecule, antibody) with ACE2 protein concentrate, then dilute the mixture with 1x Assay Diluent dilute to make a 1x ACE2 protein working concentration. Each sample should contain the same 1x ACE2 protein concentration.
Assay Procedure:	1. Bring all reagents to room temperature (18 - 25 °C) before use. It is recommended that all samples should be run in at least duplicate. 2. Add 100 µL of each sample into appropriate wells. Cover well with plate holder and incubate for 2.5 hours at room temperature or over night at 4 °C with shaking. 3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 µL) using a multi-channel pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels. 4. Add 100 µL of prepared 1x detection antibody, anti-ACE2 (Reagent Preparation step 5) to each well. Incubate for 1 hour at room temperature with shaking. 5. Discard the solution. Repeat the wash as described in Step 3. 6. Add 100 µL of prepared 1x HRP-conjugated anti-goat IgG (see Reagent Preparation Step 6) to each well. Incubate for 1 hour at room temperature with shaking. 7. Discard the solution. Repeat the wash as described in Step 3. 8. Add 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with shaking. 9. Add 50 µL of Stop Solution (Item I) to each well. Read at 450 nm immediately.
Calculation of Results:	Determine the average absorbance across the replicate readings per sample.
Restrictions:	For Research Use only

Handling

Storage:	4 °C/-20 °C
Storage Comment:	The kit may be stored at 4°C up to 1 month from the date of shipment. The life of the kit can be extended up to 6 months from the date of shipment by storing the kit reagents (Item F, C-1, and D-2) at -20°C. All other components should be stored at 4°C.
Expiry Date:	1 month

Publications

Product cited in:

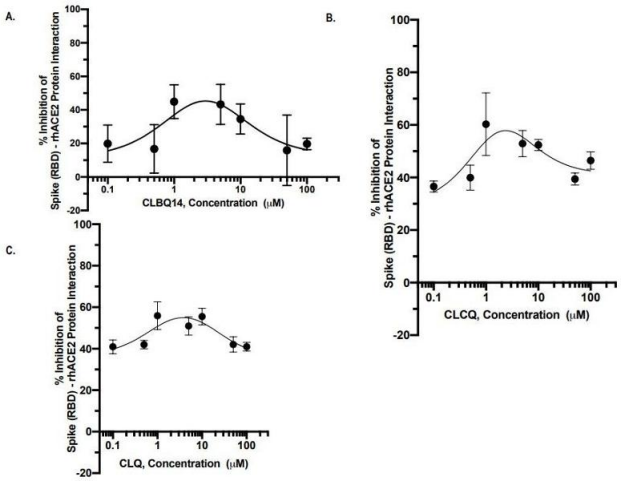
Ao, Chan, Ouyang, Olukitibi, Mahmoudi, Kobasa, Yao: "Identification and evaluation of the inhibitory effect of Prunella vulgaris extract on SARS-coronavirus 2 virus entry." in: **PLoS ONE**, Vol. 16, Issue 6, pp. e0251649, (2021) (PubMed).

Olaleye, Kaur, Onyenaka: "Ambroxol Hydrochloride Inhibits the Interaction between Severe Acute Respiratory Syndrome Coronavirus 2 Spike Protein's Receptor Binding Domain and Recombinant Human ACE2." in: **bioRxiv : the preprint server for biology**, (2020) (PubMed).

Olaleye, Kaur, Onyenaka, Adebusuyi: "Discovery of Clioquinol and Analogues as Novel Inhibitors of Severe Acute Respiratory Syndrome Coronavirus 2 Infection, ACE2 and ACE2 - Spike Protein Interaction In Vitro." in: **bioRxiv : the preprint server for biology**, (2020) (PubMed).

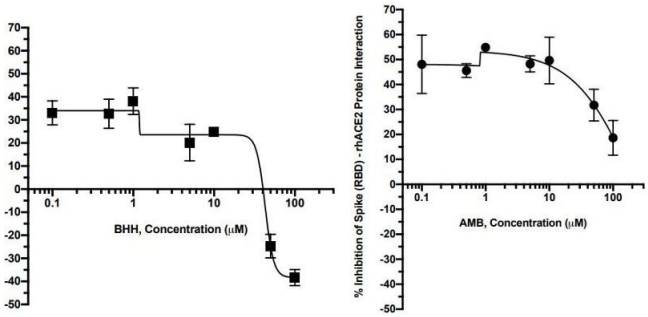
Aguilar-Pineda, Albaghdadi, Jiang, Lopez, Del-Carpio, Valdez, Lindsay, Malhotra, Lino Cardenas: " Structural and functional analysis of female sex hormones against SARS-Cov2 cell entry." in: **bioRxiv : the preprint server for biology**, (2020) (PubMed).

Images



Binding Studies

Image 1. Inhibition of rhACE2 and SARS-CoV-2 Spike (RBD) Protein Interaction by Clioquinol (CLQ) and Analogues: A. CLBQ14, B. CLCQ, and C. CLQ. Source: 10.1101/2020.08.14.250480



Binding Studies

Image 2. Effect of Bromhexine Hydrochloride (BHH) and Ambroxol Hydrochloride (AMB) on the interaction of rhACE2 with SARS-CoV-2 Spike (RBD) protein Interaction: A. BHH, and B. AMB.

Estimated Relative IC_{50} (µM) for Spike (RBD)-rhACE2 Protein Interaction Assay

Inhibitor ID	IC_{50_1} (µM)	IC_{50_2} (µM)
BHH	1.19	42.90
AMB	0.82	231.60

Binding Studies

Image 3. Activity of Ambroxol Hydrochloride (AMB) and Bromhexine Hydrochloride (BHH) against rhACE2 and SARS-CoV-2 Spike (RBD) protein Interaction. Source: 10.1101/2020.09.13.295691

Please check the product details page for more images. Overall 4 images are available for ABIN6952755.