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Description

The Spike S1 (B.1.1.529 BA.1, Omicron Variant) (SARS-CoV-2): ACE2 TR-FRET Assay is designed to measure the inhibition of the binding between SARS-CoV-2 Spike S1 (B.1.1.529 BA.1, Omicron Variant) and human ACE2 in a homogeneous 384 reaction format. This TR-FRET-based assay requires no time-consuming washing steps, making it especially suitable for high throughput screening applications. The assay procedure is straightforward and simple; the test inhibitor compound is incubated with biotinylated Spike S1, Eu-labeled ACE2, and the dye-labeled acceptor for one hour. Then the TR-FRET signal is measured using a fluorescence reader capable of measuring Time Resolved-Fluorescence Resonance Energy Transfer (TR-FRET).

Assay principle: TR-FRET homogenous assay

Background

The pandemic coronavirus disease 2019 (COVID-19) is caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). As a first step of the viral replication strategy, the virus attaches to the host cell surface before entering the cell. The Spike protein recognizes and attaches to the Angiotensin-Converting Enzyme 2 (ACE2) receptor found on the surface of type I and II pneumocytes, endothelial cells, and ciliated bronchial epithelial cells. SARS-CoV-2 Variant B.1.1.529 BA.1, also known as Omicron variant, was originally discovered in South Africa and has recently become a global variant of concern. This variant displays increased transmissibility and infectivity compared to previous known variants.

Drugs targeting the interaction between the Spike protein of SARS-CoV-2 and human ACE2 may offer some protection against the viral infection. The SARS-CoV-2 Spike (B.1.1.529 BA.1, Omicron Variant) protein in this kit consists of the S1 protein, which includes the ACE2 binding site. Compared to the wild-type strain, it contains mutations A67V, Δ69-70, T95I, G142D, Δ143-145, Δ211, L212I, Ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, K484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H.

Applications

This kit is useful to screen for inhibitors of the interaction between SARS-CoV-2 Spike S1 (Omicron Variant) and human ACE2.

Supplied Materials

Catalog #	Name	Amount	Storage
100705	ACE2, His-Tag, Eu-labeled*	2 x 2 µg	-80°C
101334	Spike S1 (B.1.1.529 BA.1, Omicron Variant), Avi-His-Tag, Biotin-Labeled (SARS-CoV-2)*	2 x 25 µg	-80°C
	Dye-labeled acceptor	3 x 10 µl	-20°C
79953	3x ACE2-Spike TR-FRET Buffer	4 ml	-20°C
79969	384-well white microplate	1	Room Temp.

****The initial concentrations of ACE2 and Spike S1 are lot-specific and will be indicated on the tubes containing the proteins.***

Materials Required but Not Supplied

Fluorescence microplate reader capable of measuring Time Resolved-Fluorescence Resonance Energy Transfer (TR-FRET)

Adjustable micropipettor and sterile tips

Storage Conditions



This assay kit will perform optimally for up to 6 months from date of receipt when the materials are stored as directed.

Safety



This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

Keep final DMSO concentration at or below 1%.

Assay Protocol

All samples and controls should be tested in duplicate.

Preparing Your Reagents

1. Thaw ACE2-Eu on ice. Upon first thaw, briefly spin the tube containing the protein to recover the full contents of the tube. Immediately store remaining undiluted protein in aliquots at -80°C . Note: ACE2-Eu is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not freeze and re-use the diluted protein.
2. Dilute one part of 3x ACE2-Spike TR-FRET Buffer with 2 parts of distilled water (3-fold dilution) to make 1x ACE2-Spike TR-FRET Buffer. Prepare only a sufficient quantity needed for the assay; store remaining stock solution in aliquots at -20°C .
3. Dilute ACE2-Eu in 1x ACE2-Spike TR-FRET Buffer to $1\text{ ng}/\mu\text{l}$ (12 nM). Keep diluted protein on ice until ready to use. Add $5\ \mu\text{l}$ of ACE2-Eu to all wells. Discard any remaining unused diluted protein after use.
4. Dilute Dye-labeled Acceptor 100-fold with 1x ACE2-Spike TR-FRET Buffer. Add $5\ \mu\text{l}$ of Dye-labeled Acceptor to all wells.
5. Prepare the Test inhibitor solution. If the inhibitor compound is water soluble (e.g. an antibody), make a solution of the compound 4-fold higher than the final concentration in 1x ACE2-Spike TR-FRET Buffer. If the inhibitor compound is a small molecule soluble in DMSO, prepare a 100x solution in DMSO, then dilute 25x in 1x ACE2-Spike TR-FRET buffer to make a 4x solution in 4% DMSO. The final DMSO concentration in the assay should be $\leq 1\%$. The diluent solution used in controls should contain the same concentration of DMSO as the test inhibitor (in this instance, 1x ACE2-Spike TR-FRET Buffer with 4% DMSO).
6. Add $5\ \mu\text{l}$ of Test inhibitor solution to each well designated "Test Inhibitor". For the "Positive Control" (no inhibitor) and "Blank", add $5\ \mu\text{l}$ of the diluent solution without inhibitor (1x ACE2-Spike TR-FRET Buffer with the same concentration of solvent as in the test inhibitor solution).

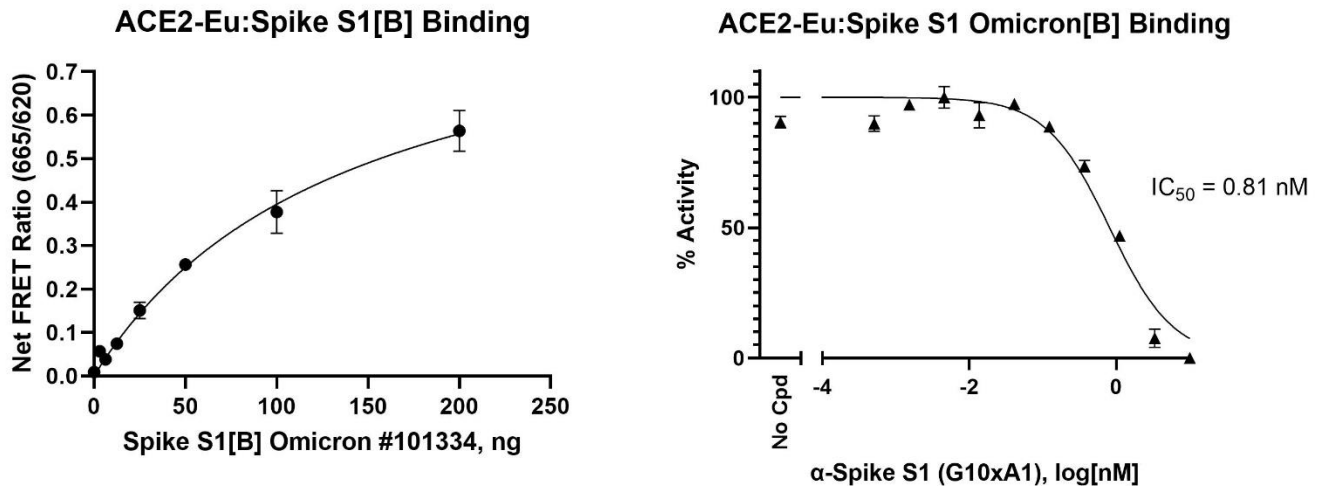
7. Thaw Spike S1-Biotin on ice. Upon first thaw, briefly spin the tube containing the protein to recover the full contents of the tube. Aliquot into single use aliquots. Immediately store remaining undiluted protein in aliquots at -80°C. Note: Spike S1-Biotin is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not freeze and re-use the diluted protein.
8. Dilute Spike S1-Biotin in 1x ACE2-Spike TR-FRET Buffer to 20 ng/μl (200 nM). Keep diluted protein on ice until ready to use. Discard any remaining unused diluted protein after use. Add 5 μl of diluted Spike S1-Biotin to wells designated "Test Inhibitor" and "Positive Control". Add 5 μl of 1x ACE2-Spike TR-FRET Buffer to wells designated "Blank".

Component	Positive Control	Blank	Test Inhibitor
ACE2-Eu (1 ng/μl)	5 μl	5 μl	5 μl
Dye-labeled acceptor	5 μl	5 μl	5 μl
Test Inhibitor			5 μl
Diluent solution (no inhibitor)	5 μl	5 μl	
1x TR-FRET Buffer		5 μl	
Spike S1-Biotin (Omicron Variant) (20 ng/μl)	5 μl		5 μl
Total	20 μl	20 μl	20 μl

9. Incubate the plate at room temperature for 1 hour.
10. Read TR-FRET signal in a microtiter-plate reader under settings described below (settings may need optimization depending on the instrument). Blank value is subtracted from all other values.

Channel	Variable	Recommended Value
1	Excitation wavelength (nm)	340 ± 20
	Emission wavelength (nm)	620 ± 10
	Lag time (μs)	60
	Integration time (μs)	500
2	Excitation wavelength (nm)	340 ± 20
	Emission wavelength (nm)	620 ± 10
	Lag time (μs)	60
	Integration time (μs)	500

Example Results



Titration of SARS-CoV-2 Spike S1 (B.1.1.529 BA.1, Omicron Variant)-Biotin (BPS Bioscience, # 101334) (left) and inhibition of Spike S1:ACE2 binding using increasing concentrations of Spike Neutralizing Antibody (Clone G10xA1; BPS Bioscience, #101326) (right) in the Spike S1-Biotin (B.1.1.529, Omicron Variant) (SARS-CoV-2):ACE2 TR-FRET Assay Kit (BPS Bioscience, #78358). Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

General Considerations

“Blank” Control: The “Blank” control is important to determine the background FRET signal in the assay. We recommend doing these in duplicate.

“Positive Control”:

The “Positive Control” is the maximum signal determined upon the addition of diluent solution (for example, 1% DMSO in 1x ACE2-Spike TR-FRET Buffer) in the absence of inhibitor.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

Reference

- Hoffmann, M. *et al.* 2020. *Cell*, **181**:1-10
- Yan, R. *et al.* 2020. *Science* **367(6485)**:1444-1448

Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
Spike S1-Biotin (SARS-CoV-2): ACE2 TR-FRET Assay Kit	79949	96/384 rxns
Spike S1 RBD (SARS-CoV-2): ACE2 Inhibitor Screening Assay Kit	79931	96 rxns
ACE2: Spike S1 RBD (SARS-CoV-2) Inhibitor Screening Assay Kit	79936	96 rxns
Spike S1 RBD (B.1.1.529 BA.1, Omicron Variant) (SARS-CoV-2): ACE2 Inhibitor Screening Chemiluminescence Assay Kit	78357	96 rxns
Spike S1 RBD (B.1.1.529, Omicron): ACE2 Inhibitor Screening Colorimetric Assay Kit	78339	96 rxns