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Data sheet

SARS-CoV-1 Spike Trimer (S1+S2):ACE2 Inhibitor Screening Colorimetric Assay Kit

Catalog #78012
Size: 96 reactions

DESCRIPTION: Severe acute respiratory syndrome (SARS) is a viral respiratory illness caused by a corona virus called SARS-CoV-1. It was first reported in February 2013 in Asia and the epidemic affected ~ 26 countries and resulted in more than 8000 deaths. Like SARS-CoV-2, SARS-CoV-1 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. Drugs targeting the interaction between the Spike protein of SARS-CoV-1 and ACE2 may offer some protection against the viral infection. The **SARS-CoV-1 Spike Trimer (S1+S2):ACE2 Inhibitor Screening Assay Kit** includes the SARS-CoV-1 Spike protein in its native trimeric conformation to provide a more physiologically relevant screen for inhibitors as well as a compatible platform to investigate the specificity of SARS-CoV-2:ACE2 inhibitors.

The **SARS-CoV-1 Spike Trimer (S1+S2):ACE2 Inhibitor Screening Assay Kit** is designed for screening and profiling inhibitors of this interaction. This kit comes in a convenient 96-well format, with **Biotinylated-ACE2**, purified **SARS-CoV-1 Spike Trimer (S1+S2) protein**, **Streptavidin-HRP**, and **assay buffers** for 100 binding reactions. The key to this kit is that the SARS-CoV-1 Spike Trimer (S1+S2) protein provides a more biologically relevant model than monomeric Spike RBD protein for the investigation of SARS-CoV-1/host cell interaction. Only a few simple steps on a microtiter plate are required for the assay. First, SARS-CoV-1 Spike Trimer (S1+S2) is coated on a 96-well plate. Next, Biotin-ACE2 is incubated with SARS-CoV-1 Spike Trimer (S1+S2) on the plate. Finally, the plate is treated with Streptavidin-HRP followed by addition of a colorimetric HRP substrate to produce color, which then can be quenched and measured using a UV/Vis microplate reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
100789	Spike Trimer (S1 + S2), His-Tag (SARS-CoV-1)*	5 µg	-80°C	Avoid multiple freeze/ thaw cycles!
100665	ACE2, His-Avi-Tag, Biotin labeled*	5 µg	-80°C	
79311	3x Immuno Buffer 1	50 ml	-20°C	
79728	Blocking Buffer 2	50 ml	+4°C	
79742	Streptavidin-HRP	5 µl	+4°C	
	Colorimetric HRP substrate	10 ml	+4°C	
79964	Transparent 96-well microplate	1	Room Temp	

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***The initial concentration of spike trimer and ACE2 is lot-specific and will be indicated on the tube containing the proteins.*

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
PBS (Phosphate buffered saline)
PBST (Phosphate buffered saline containing 0.05% Tween-20)
1N HCl (aqueous)
Rotating or rocker platform
UV/Vis spectrophotometer microplate reader capable of reading absorbance at 450 nm*

APPLICATIONS: This kit is useful for screening for inhibitors of ACE2 binding to trimeric SARS-CoV-1 Spike

STABILITY: Up to 6 months from date of receipt, when stored as recommended.

REFERENCES:

Yan, R. *et al.* 2020. *Science* **367 (6485):** 1444-1448.
Li, W., *et al.* 2003. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* **426:**450-454.
Li, J., *et al.* 2013. Immunogenicity and Protection Efficacy of Monomeric and Trimeric Recombinant SARS Coronavirus Spike Protein Subunit Vaccine Candidates. *Viral Immunol.* **26(2):** 126–132.

ASSAY PROTOCOLS:

Protocol 1:
Inhibition of ACE2:SARS-CoV-1 Spike binding by an anti-SARS-CoV-1 Spike antibody

All samples and controls should be tested in duplicate.

Coating the plate with SARS-CoV-1 Spike Trimer (S1+S2) protein:

1) Thaw **Spike Trimer (S1+S2) protein** on ice. Upon first thaw, briefly spin tube containing **Spike Trimer (S1+S2) protein** to recover the full contents of the tube. Aliquot into single use aliquots. Immediately store remaining **Spike Trimer (S1+S2) protein** in aliquots at -80°C. Note: **Spike protein** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.
2) Dilute **Spike Trimer (S1+S2) protein** to 1 µg/ml in PBS.
3) Add 50 µl of diluted **Spike Trimer (S1+S2) protein** solution to each well and incubate at 4ºC overnight.

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4) After the overnight coating, discard the solution and wash the plate three times with 150 µl PBST. Tap plate onto clean paper towels to remove liquid.

5) Block wells by adding 200 µl **Blocking Buffer 2** to each well. Incubate for 1 hour at room temperature with slow shaking. Remove the blocking solution and wash the plate with 150 µl PBST three times. Tap the plate onto clean paper towels to remove liquid.

Step 1

1) Prepare **1x Immuno Buffer** by diluting **3x Immuno Buffer** in distilled water (one portion of **3x Immuno Buffer** is added to two portions of distilled water).

2) Dilute an anti-Spike antibody in **1x Immuno Buffer** at 2x desired concentration (If there is no information how potent the antibody is, it is recommended to use serial dilutions of the antibody. If the antibody is already diluted in serum or cell culture medium, further dilution may not be required).

3) Add 25 µl of the diluted antibody to the wells labeled "Test Inhibitor." To the wells labeled "Blank" and "Positive Control," add 25 µl **1x Immuno Buffer**. Incubate the plate for 1 hour at room temperature with slow rotation.

4) Thaw the **Biotin-ACE2** on ice, and dilute it at 2 ng/µl in **1x Immuno Buffer**. Prepare only the amount required for the assay; store remaining **Biotin-ACE2** undiluted at -80°C. Note: **Biotin-ACE2** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.

5) After 1 hour incubation of the antibody, add 25 µl of diluted **Biotin-ACE2** to the wells labeled "Test Inhibitor" and "Positive Control." Add 25 µl **1x Immuno Buffer** to the wells labeled "Blank." Incubate the plate at room temperature for another 1 hour with slow rotation.

	Blank	**Positive Control**	**Test Inhibitor**
1x Immuno Buffer 1	50 µl	25 µl	-
Test antibody	-	-	25 µl
ACE2-Biotin (2.0 ng/µl)	-	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**

6) After 1 hour, discard the solution and wash the plate three times with 150 µl PBST. Tap the plate onto clean paper towels to remove liquid.

Step 2

1) Dilute **Streptavidin-HRP** 1000-fold with the **Blocking Buffer 2**

2) Add 50 µl of the **diluted Streptavidin-HRP** to each well and incubate the plate for 30 min at room temperature with slow rotating.

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3) After 30 minutes, discard the solution and wash the plate three times with 150 ul PBST. Tap the plate onto clean paper towel to remove liquid.

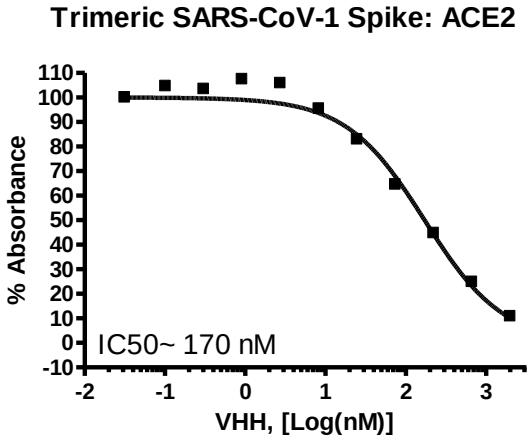
4) Prepare 1M HCl(aq) stop solution. *Note: alternatively, 2N H_2SO_4 or other compatible acidic solutions can be substituted.*

5) Add 100 µl of the **Colorimetric HRP substrate** to each well and incubate the plate at room temperature until blue color is developed in the "Positive Control" wells. This usually takes a few minutes but may develop faster. The optimal incubation time may vary, and should be determined empirically by the user. It is recommended that the reaction be stopped when the 'Positive Control' well is lower than ~ 1.0 absorbance at 450 nm (preferably ~ 0.6).

6) Once blue color is developed in the "Positive Control" well, add 100 µl stop solution prepared above. The "Positive Control" well should appear yellow.

7) Read the absorbance at 450 nm using UV/Vis spectrophotometer microplate reader.

Example of assay results:



Inhibition of ACE2:SARS-CoV-1 Spike binding by a small anti-SARS-CoV-1 Spike antibody, VHH. A small anti-SARS-CoV-1 Spike antibody was evaluated using the ***SARS-CoV-1 Spike Trimer (S1+S2):ACE2 Inhibitor Screening Colorimetric Assay Kit***. The antibody was serially diluted in 1x Immuno Buffer and tested following the assay kit protocol.

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Protocol 2:
Inhibition of ACE2:SARS-CoV-1 Spike binding by a small molecule inhibitor

All samples and controls should be tested in duplicate.

Coating the plate with SARS-CoV-1 Spike Trimer (S1+S2) protein:

Coat the plate as described above.

Step 1

1) Prepare 1x Immuno Buffer by diluting 3x Immuno Buffer in distilled water (one portion of 3x Immuno Buffer is added to two portions of distilled water). Add 25 µl 1x Immuno Buffer to each well.

2) Prepare the test inhibitor in DMSO (or distilled water if soluble), and further dilute it in distilled water at 10X testing concentration. (*e.g.* To test a compound at 10 µM, prepare the inhibitor in DMSO at 1 mM. Then make a 10-fold dilution distilled water to create a 100 µM solution in 10% DMSO(aq)).

3) Add 5 µl to each well labeled "Test Inhibitor." To the "Positive Control" and "Blank" wells, add 5 µl of the same solution without inhibitor (e.g. 10% DMSO(aq) solution) so that all wells contain the same amount of DMSO. *Caution! – It is highly recommended that the final DMSO concentration should not exceed 1%. Organic solvents other than DMSO are not validated in this assay, so use of these solvents must be optimized by the user*.

4) Thaw the **Biotin-ACE2** on ice, and dilute it in 1x Immuno Buffer at 2.0 ng/µl. Prepare only the amount required for the assay; store remaining **Biotin-ACE2** undiluted at -80°C. Note: **Biotin-ACE2** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.

5) Add 20 µl 1x Immuno Buffer to the wells labeled "Blank". Add 20 µl of diluted **Biotin-ACE2** to the wells labeled "Test Inhibitor" and "Positive Control." Incubate the plate at room temperature for 1 hour with slow rotation.

	Blank	Positive Control	Test Inhibitor
1x Immuno Buffer 1	45 µl	25 µl	25 µl
Test Inhibitor	-	-	5 µl
Inhibitor solution (no inhibitor) – usually 10% DMSO(aq)	5 µl	5 µl	-
ACE2-Biotin (2.0 ng/µl)	-	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**

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6) After 1 hour, discard the solution and wash the plate three times with 150 µl PBST. Tap the plate onto clean paper towels to remove liquid.

Step 2

7) Dilute **Streptavidin-HRP** 1000-folds with the Blocking Buffer 2

8) Add 50 µl of the **diluted Streptavidin-HRP** to each well and incubate the plate for 30 minutes at room temperature with slow rotation.

9) After 30 minutes, discard the solution and wash the plate three times with 150 ul PBST. Tap the plate onto clean paper towel to remove liquid.

10) Prepare 1M HCl(aq) stop solution. *Note: alternatively, 2N H_2SO_4 or other compatible acidic solutions can be substituted.*

11) Add 100 µl of the **Colorimetric HRP substrate** to each well and incubate the plate at room temperature until blue color is developed in the "Positive Control" well. This usually takes a few minutes but may develop faster. The optimal incubation time may vary, and should be determined empirically by the user. It is recommended that the reaction be stopped when the "Positive Control" well is lower than ~ 1.0 absorbance at 450 nm (preferably ~ 0.6).

12) Once blue color is developed in the positive well, add 100 µl stop solution prepared above. The "Positive Control" well should appear yellow.

13) Read the absorbance at 450 nm using UV/Vis spectrophotometer microplate reader.

RELATED PRODUCTS:

Product Name	Catalog#	Size
Spike Trimer (S1+S2), His-tag (SARS-CoV)	100789	100 µg/500 µg
Spike Trimer (S1+S2), His-tag (HCoV-NL63)	100788	100 µg/500 µg
Spike Trimer (S1+S2), His-tag (SARS-CoV-2)	100728	100 µg/1 mg
SARS-CoV-2 Spike:ACE2 Inhibitor Screening Assay Kit	79931	96 reactions
ACE2:SARS-CoV-2 Spike Inhibitor Screening Assay Kit	79936	96 reactions
ACE2:SARS-CoV-2 Spike S1-Biotin Inhibitor Screening Assay Kit	79945	96 reactions
SARS-CoV-2 Spike S1-Biotin:ACE2 TR-FRET Assay Kit	79949	96 reactions
Spike S1, Fc Fusion, Avi-tag (SARS-CoV-2)	100678	100 µg/1 mg
Spike S1, Fc fusion, Avi-tag, Biotin-Labeled (SARS-CoV-2)	100679	25 µg/50 µg
Spike S1 RBD, His-tag (SARS-CoV-2)	100687	50 µg/100 µg
Spike S1, Fc fusion (SARS-CoV-2)	100688	20 µg/50 µg
Spike S1 RBD, Fc fusion (SARS-CoV-2)	100699	50 µg/100 µg
ACE2 Inhibitor Screening Assay Kit	79923	96 reactions
ACE2, His-tag	11003	20 µg/100 µg

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ACE2, His-Avi-Tag, Biotin-labeled HiP™ 100665 20 µg/50 µg

TROUBLESHOOTING GUIDE:

Problem	Possible cause	Solution
Luminescence signal of positive control reaction is weak	Spike and ACE2-Biotin have lost activity	Proteins lose activity upon repeated freeze/thaw cycles. Use fresh proteins. Store proteins in single-use aliquots. Increase time of enzyme incubation. Increase enzyme concentration.
	Incorrect settings on instruments	Refer to instrument instructions for settings to increase sensitivity of light detection.
	Colorimetric HRP substrate was not incubated long enough	Increase the amount of time that the colorimetric HRP substrate is incubated in the wells. Avoid azides.
Colorimetric signal is erratic or varies widely among wells	Inaccurate pipetting/technique	Run duplicates of all reactions. Use a multichannel pipettor. Use master mixes to minimize errors.
	Bubbles in wells	Pipette slowly to avoid bubble formation. Tap plate lightly to disperse bubbles; be careful not to splash between wells.
	Signal is out of range of detection (too high)	Decrease the amount of time that the colorimetric HRP substrate is incubated in the wells
Background (signal to noise ratio) is high	Insufficient washes	Increase number of washes. Increase wash volume.
	Sample solvent is inhibiting the enzyme	Run negative control assay including solvent. Maintain DMSO level at <1% Increase time of enzyme incubation.
	Results are outside the linear range of the assay	Use different concentrations of proteins to create a standard curve