ACE2/SPIKE(N354D,D364Y) BINDING ASSAY KITS

PROTOCOL

Part # 63ADK000CB25PEG & 63ADK000CB25PEH

Test size: 500 tests (63ADK000CB25PEG), 10,000 tests (63ADK000CB25PEH) - assay volume: 20 µL

Revision: 01 (June 2020)

Store at: -60°C or below

This product is intended for research purposes only. The product is not intended to be used for therapeutic or diagnostic purposes.

ASSAY PRINCIPLE

The HTRF SARS-CoV-2 Spike (N354D, D364Y)/ACE2 Binding Assay is designed to measure the interaction between SARS-CoV-2 Spike (N354D, D364Y) protein RBD and human ACE2 proteins. Utilizing HTRF (Homogeneous Time-resolved Fluorescence) technology, the assay enables simple and rapid characterization of compound and antibody blockers in a high throughput format.

As shown in Figure 1, the interaction between Tag1-SARS-CoV-2 Spike (N354D, D364Y) and Tag2-ACE2 is detected by using anti-Tag1-Europium (HTRF donor) and anti-Tag2-d2 (HTRF acceptor). When the donor and acceptor antibodies are brought into close proximity due to SARS-CoV-2 Spike (N354D, D364Y) and ACE2 binding, excitation of the donor antibody triggers fluorescent resonance energy transfer (FRET) towards the acceptor antibody, which in turn emits specifically at 665 nm. This specific signal is directly proportional to the extent of SARS-CoV-2 Spike (N354D, D364Y)/ACE2 interaction. Thus, compound or antibody blocking SARS-CoV-2 Spike (N354D, D364Y)/ACE2 interaction will cause a reduction in HTRF signal.

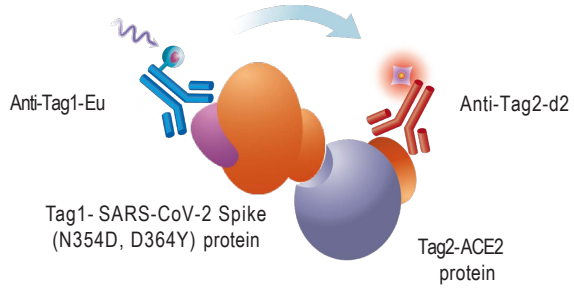
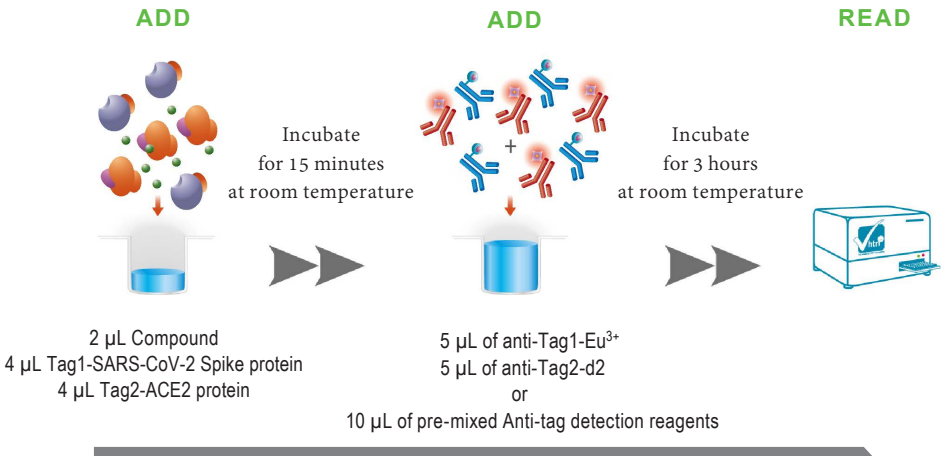


Figure 1: Principle of the HTRF SARS-CoV-2 Spike (N354D, D364Y)/ACE2 assay.

PROTOCOL AT A GLANCE



Make sure to use the setup for Eu^{3+} Cryptate. For more information about set-up and compatible HTRF® readers, please visit our website at: http://www.cisbio.com/readers

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INTERACTION IS EVERYTHING

MATERIALS:

KIT COMPONENTS	500 TESTS CAT # 63ADK000CB25PEG	10,000 TESTS CAT # 63ADK000CB25PEH
Tag1-SARS-CoV-2 Spike (N354D, D364Y)* MW: 27.0 kDa	1 vial Frozen see concentration and volume on vial label	1 vial Frozen see concentration and volume on vial label
Tag2-ACE2* MW: 111.7 kDa	1 vial Frozen see concentration and volume on vial label	1 vial Frozen see concentration and volume on vial label
Anti-Tag1-Eu^{3+}	1 vial 25 µL 100 X Frozen	1 vial 0.5 mL 100 X Frozen
Anti-Tag2-d2	1 vial 25 µL 100 X Frozen	1 vial 0.5 mL 100 X Frozen
Diluent	1 vial 20 mL Cat# 62DLBDDF (200 mL) ready-to-use	1 vial 200 mL Cat# 62DLBDDF ready-to-use
Detection Buffer	1 vials 10 mL Cat# 62DB1FDG (130 mL) ready-to-use	1 vial 130 mL Cat# 62DB1FDG (130 mL) ready-to-use

* The amounts of Tag1-SARS-CoV-2 Spike (N354D, D364Y) and Tag2-ACE2 provided are sufficient for the validated amounts of tagged proteins suitable for compound inhibition study: 2.5 nM of SARS-CoV-2 Spike (N354D, D364Y) and 7.5 nM of ACE2 in 20 µL final assay volume.

For reading, an HTRF®-Certificated Reader is needed.

For HTRF microplate recommendations, please visit http://www.cisbio.com/microplate-recommendations
For a list of HTRF-compatible readers and setup recommendations, please visit http://www.cisbio.com/readers

STORAGE AND STABILITY

Store the kit at -60°C or below. Under appropriate storage conditions, reagents are stable until the expiry date indicated on the label.

Reagents

Once thawed, tagged SARS-CoV-2 Spike & ACE2 stock solution may be frozen, and can be thawed only once. Once thawed (or reconstituted), anti-Tag solutions can be frozen once.
To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -60°C or below.

Thawed diluent and detection buffer can be stored at 2-8°C on your premises.

REAGENT PREPARATION

BEFORE YOU BEGIN:

- It is very important to prepare reagents in the specified buffers. The use of an incorrect diluent may affect reagent stability and assay results.
- Thaw the frozen reagents at room temperature.
- Before use, allow all reagents to warm up to room temperature then homogeneize buffer and diluent. It is recommended to filter buffers before use.
- The tagged protein solutions must be prepared in individual vials - DO NOT premix tagged solutions prior to dispensing.
- The anti-Tag solutions must be prepared in individual vials and can be premix prior to dispensing.
- Compounds may be prepared in diluent. We recommend keeping DMSO below 0.5% during the assay (20 µL final volume).

TO PREPARE WORKING SOLUTIONS:

Take care to prepare stock and working solutions according to the directions for the kit size you have purchased.

500 TESTS	10,000 TESTS
Tag1-SARS-CoV-2 Spike (N354D, D364Y) protein Concentration and volume are indicated on the vial label	
Thaw the Tag1-SARS-CoV-2 Spike (N354D, D364Y) protein* solution. Prepare working solutions in diluent which have 5 X the required final concentration for binding assay*: e.g. Prepare a 12.5 nM Tag1-SARS-CoV-2 Spike working solution for a final concentration of 2.5 nM Tag1-SARS-CoV-2 Spike (20 µL final volume).	
Tag2-ACE2 protein Concentration and volume are indicated on the vial label	
Thaw the Tag2-ACE2 protein* solution. Prepare working solutions in diluent which have 5 X the required final concentration for binding assay*. e.g. Prepare a 37.5 nM Tag2-ACE2 working solution for a final concentration of 7.5 nM Tag2-ACE2 (20 µL final volume).	
Anti-Tag1-Eu^{3+}	
Thaw the anti-Tag1-Eu^{3+} solution. This 100 X stock solution can be frozen and stored at -60°C or below. Dilute 100-fold the 100 X anti-Tag1-Eu^{3+} stock solution with detection buffer.	
e.g. 25 µL of thawed anti-Tag1-Eu^{3+} stock solution + 2475 µL of detection buffer.	e.g. 0.5 mL of thawed anti-Tag1-Eu^{3+} stock solution + 49.5 mL of detection buffer.
Anti-Tag2-d2	
Thaw the anti-Tag2-d2 solution. This 100 X stock solution can be frozen and stored at -60°C or below. Dilute 100-fold the 100 X anti-Tag2-d2 stock solution with detection buffer.	
Dilute 100-fold the 100 X anti-Tag2-d2 stock solution with detection buffer. e.g. 25 µL of thawed anti-Tag2-d2 stock solution + 2475 µL of detection buffer.	Dilute 100-fold the 100 X anti-Tag2-d2 stock solution with detection buffer. e.g. 0.5 mL of reconstituted anti-Tag2-d2 stock solution + 49.5 mL of detection buffer.

*Titration of Tag1-SARS-CoV-2 Spike (N354D, D364Y) or Tag2-ACE2 can be performed if necessary.

ASSAY PROTOCOL

Step 1		Dispense 2 µL of compound/antibody or diluent 4 µL of Tag1-SARS-CoV-2 Spike protein 4 µL of Tag2-ACE2 protein.
Step 2		Incubate for 15 minutes at room temperature.
Step 3		Dispense 10 µL of pre-mixed anti-Tag1-Eu^{3+} and anti-Tag2-d2.
Step 4		Seal the plate and incubate for 3 hours at room temperature.
Step 5		Remove the plate sealer and read on an HTRF® compatible reader.

STANDARD PROTOCOL FOR INHIBITORY ASSAY IN 20 µL FINAL VOLUME

	Inhibitor	Tag1-SARS-CoV-2 Spike	Tag2-ACE2	Anti-Tag1-Eu^{3+}	Anti-Tag2-d2	Diluent	Detection buffer
Sample	2 µL	4 µL	4 µL	5 µL	5 µL		
Positive control		4 µL	4 µL	5 µL	5 µL	2 µL	
Negative control		4 µL		5 µL	5 µL	6 µL	
Cryptate control				5 µL		10 µL	5 µL
Buffer control						10 µL	10 µL

EXAMPLE OF PLATE MAP

	1	2	3	4	5	6
A	**Buffer control**: 10 µL diluent 10 µL detection buffer	Repeat Well A1	Repeat Well A1	**Compound...:** 2 µL compound... 4 µL Tag1-SARS-CoV-2 Spike 4 µL Tag2-ACE2 10 µL pre-mix anti-Tag reagents	Repeat Well A4	Repeat Well A4
B	**Cryptate control:** 10 µL diluent 5 µL detection buffer 5 µL anti-Tag1-Eu	Repeat Well B1	Repeat Well B1	**Compound...:** 2 µL compound... 4 µL Tag1-SARS-CoV-2 Spike 4 µL Tag2-ACE2 10 µL pre-mix anti-Tag reagents	Repeat Well B4	Repeat Well B4
C	**Negative control:** 6 µL diluent 4 µL Tag1-SARS-CoV-2 Spike 10 µL pre-mix anti-Tag reagents	Repeat Well C1	Repeat Well C1	**Compound...:** 2 µL compound... 4 µL Tag1-SARS-CoV-2 Spike 4 µL Tag2-ACE2 10 µL pre-mix anti-Tag reagents	Repeat Well C4	Repeat Well C4
D	**Positive control:** 2 µL diluent 4 µL Tag1-SARS-CoV-2 Spike 4 µL Tag2-ACE2 10 µL pre-mix anti-Tag reagents	Repeat Well D1	Repeat Well D1	**Compound...:** 2 µL compound... 4 µL Tag1-SARS-CoV-2 Spike 4 µL Tag2-ACE2 10 µL pre-mix anti-Tag reagents	Repeat Well D4	Repeat Well D4
E	**Compound 1:** 2 µL compound 1 4 µL Tag1-SARS-CoV-2 Spike 4 µL Tag2-ACE2 10 µL pre-mix anti-Tag reagents	Repeat Well E1	Repeat Well E1	**Compound...:**	Repeat Well E4	Repeat Well E4
F	**Compound 2:** 2 µL compound 2 4 µL Tag1-SARS-CoV-2 Spike 4 µL Tag2-ACE2 10 µL pre-mix anti-Tag reagents	Repeat Well F1	Repeat Well F1	**Compound...:**	Repeat Well F4	Repeat Well F4
G	**Compound...:** 2 µL compound... 4 µL Tag1-SARS-CoV-2 Spike 4 µL Tag2-ACE2 10 µL pre-mix anti-Tag reagents	Repeat Well G1	Repeat Well G1	**Compound...:**	Repeat Well G4	Repeat Well G4
H	**Compound...:** 2 µL compound... 4 µL Tag1-SARS-CoV-2 Spike 4 µL Tag2-ACE2 10 µL pre-mix anti-Tag reagents	Repeat Well H1	Repeat Well H1			

DATA REDUCTION & INTERPRETATION

1. Calculate the ratio of the acceptor and donor emission signals for each individual well.

$$\text{Ratio} = \frac{\text{Signal 665 nm}}{\text{Signal 620 nm}} \times 10^4$$

2. Calculate the % CVs. The mean and standard deviation can then be worked out from ratio replicates.

$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

For more information about data reduction, please visit http://www.cisbio.com/data-reduction

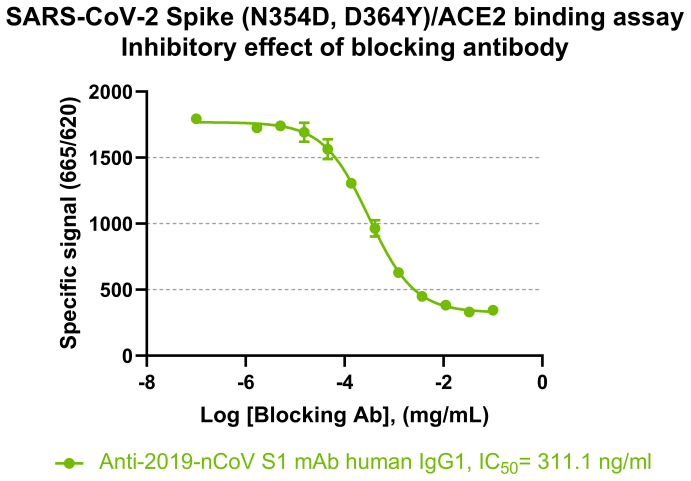
RESULTS

The data shown below must not be substituted for the data obtained in the laboratory, and should be considered only as an example.

The inhibitory effect of human IgG1 neutralizing antibody of SARS-CoV-2 was tested at 2.5 nM Tag1-SARS-CoV-2 Spike (N354D, D364Y) and 7.5 nM ACE2.

Readouts on VICTOR Nivo with a flash lamp.

Note that results may vary from one HTRF® compatible reader to another.



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