S1/ACE2 BINDING ASSAY KITS

PROTOCOL

Part # 64BDS1PEG & 64BDS1PEH

Test size: 500 tests (64BDS1PEG), 10 000 tests (64BDS1PEH) - assay volume: 20 µL

Revision: 02 - Jan 2021

Store at: ≤-60°C

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 S1/ACE2 Binding Assay is designed to measure the interaction between S1 and ACE2. 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, ACE2 is labeled with Europium (HTRF donor) and S1 is labeled with d2 (HTRF acceptor). When the donor and acceptor are brought into close proximity due to S1 and ACE2 binding, excitation of the donor triggers fluorescence resonance energy transfer (FRET) towards the acceptor, which in turn emits specifically at 665 nm. This specific signal is directly proportional to the extent of S1/ACE2 interaction. Thus, compound or antibody blocking S1/ACE2 interaction will cause a reduction in HTRF signal

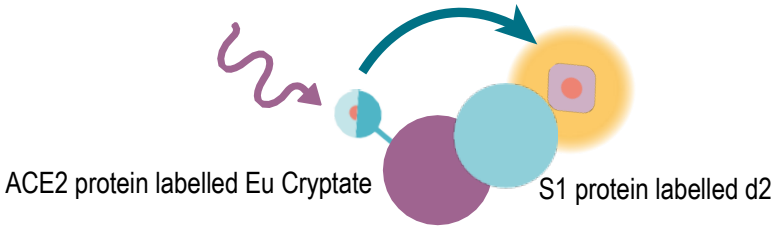
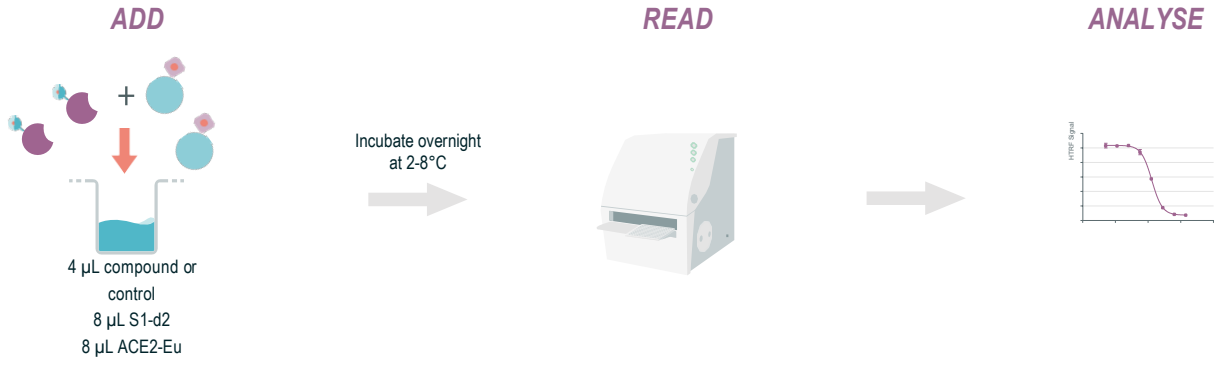


Figure 1: Principle of the HTRF S1/ACE2 assay

PROTOCOL AT A GLANCE



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MATERIALS:

KIT COMPONENTS	500 TESTS CAT # 64BDS1PEG	10,000 TESTS CAT # 64BDS1PEH
ACE2 protein Eu Cryptate- Frozen	1 vial - 80 µL 50X	1 vial – 1600 µL 50X
S1 protein d2 - Frozen	1 vial - 80 µL 50X	1 vial – 1600 µL 50X
Control kit	1 vial 50 µL 500nM	1 vial 50 µL 500nM
Detection Buffer Frozen	1 vial - 50 mL	3 vials - 105 mL

For reading, an HTRF®-Certified Reader is needed. Make sure to use the set-up for Eu Cryptate. For a list of HTRF-compatible readers and setup recommendations, please visit our website at: www.cisbio.com/readers

For HTRF microplate recommendations, please visit:www.cisbio.com/microplate-recommendations

STOR AGE AND STABILITY

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

Reagents

To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at ≤-60°C.

Thawed detection Buffer can be stored at 2-8°C on your premises.

REAGENT PREPAR ATION

BEFORE YOU BEGIN:

- It is very important to prepare reagents in the specified detection buffer. The use of an incorrect buffer 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 detection buffer. It is recommended to filter buffers before use.
- The labelled proteins solutions must be prepared in individual vials.
- Compounds may be prepared in the specified detection buffer.

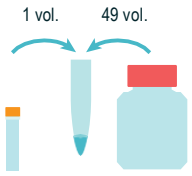
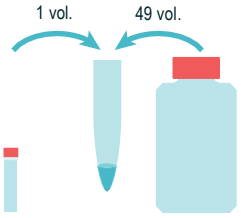
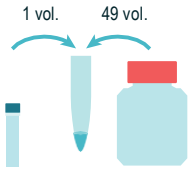
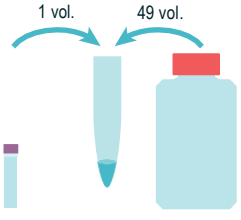
TO PREPARE STOCK SOLUTIONS:

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

500 TESTS		10,000 TESTS	
Control			
Thaw the control. Mix gently. This control stock solution can be frozen and stored at ≤ -60°C.			Thaw the control. Mix gently. This control stock solution can be frozen and stored at ≤ -60°C..
ACE2 Eu Cryptate			
Thaw the ACE2 Eu Cryptate. Mix gently. This 50X ACE2 Eu Cryptate stock solution can be frozen and stored at ≤ -60°C.			Thaw the ACE2 Eu Cryptate. Mix gently. This 50X ACE2 Eu Cryptate stock solution can be frozen and stored at ≤ -60°C.
S1 d2			
Thaw the S1 d2. Mix gently. This 50X S1 d2 stock solution can be frozen and stored at ≤ -60°C.			Thaw the S1 d2. Mix gently. This 50X S1 d2 stock solution can be frozen and stored at ≤ -60°C.
Detection Buffer			
Thaw the detection Buffer The thawed buffer can be stored at 2-8°C on your premises.			Thaw the detection Buffer The thawed buffer can be stored at 2-8°C on your premises.

TO PREPARE ACE2 EU CRYPTATE AND S1 D2 WORKING SOLUTIONS:

Each well requires 8 µL of each protein donor & acceptor reagents.

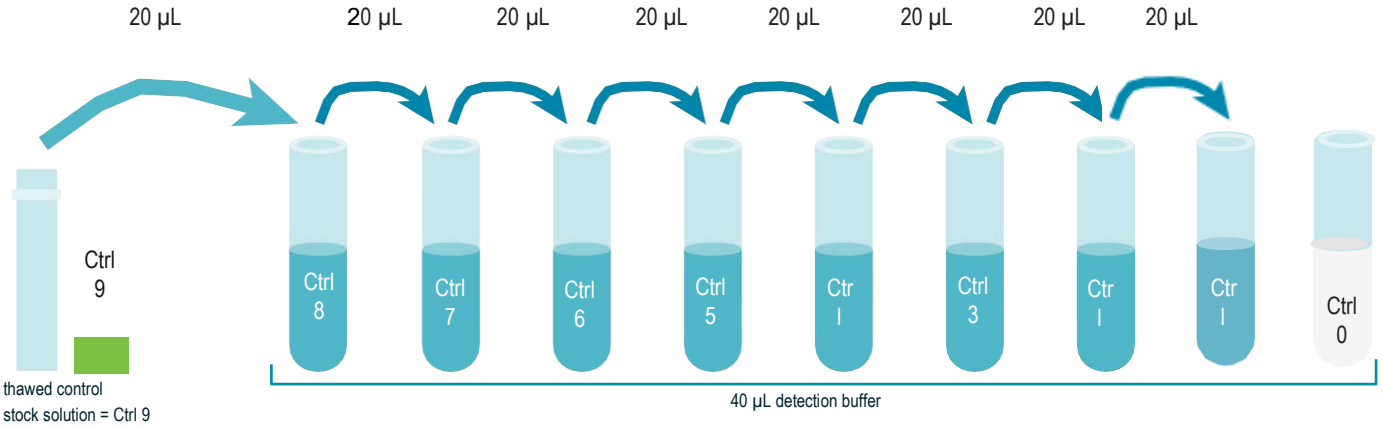
500 TESTS		10,000 TESTS	
ACE2 Eu Cryptate			
Dilute 50-fold the 50X stock solution (thawed reagent) of ACE2 Eu Cryptate with Detection Buffer : e.g. 80 µL of ACE2 Eu Cryptate stock solution + 3920 µL of Detection Buffer.			Dilute 50-fold the 50X stock solution (thawed reagent) of ACE2 Eu Cryptate with Detection Buffer : e.g. 1600 µL of ACE2 Eu Cryptate stock solution + 78400 µL of Detection Buffer.
S1 d2			
Dilute 50-fold the 50X stock solution (thawed reagent) of S1 d2 with Detection Buffer : e.g. 80 µL of S1 d2 stock solution + 3920 µL of Detection Buffer.			Dilute 50-fold the 50X stock solution (thawed reagent) of S1 d2 with Detection Buffer : e.g. 1600 µL of S1 d2 stock solution + 78400 µL of Detection Buffer.

TO PREPARE WORKING CONTROL SOLUTIONS:

- Each well requires 4 µL of control.
- In order to counteract any control sticking, we recommend changing tips between each dilution.

A recommended control dilution procedure is listed and illustrated below:

1. Thaw the control vial, the concentration of the control stock solution = 500 nM
2. Prepare the following dilutions:
   - •Control stock solution are the high control (Ctrl9: 500nM) for the top of the curve.
   - •.Use the high control (Ctrl9) to prepare the control curve using 3-fold serial dilutions as follows:
     - Dispense 40 µL of detection buffer into each vial from Ctrl8 to Ctrl0
     - Add 20 µL of control to 40 µL of detection buffer, mix gently and repeat the serial dilution to make control solutions: ctrl8, ctrl7, ctrl6, ctrl5, ctrl4, ctrl3, ctrl2, ctrl1.
       This will create 9 controls. Ctrl 0 is detection buffer alone.



CONTROL	SERIAL DILUTIONS	WORKING SOLUTIONS	FINAL CONCENTRATIONS
Control Stock solution = Control 9	Thaw the control stock solution	500 nM	100 nM
Control 8	20 µL Control 9 + 40 µL detection buffer	166.7 nM	33 nM
Control 7	20 µL Control 8 + 40 µL detection buffer	55.6 nM	11 nM
Control 6	20 µL Control 7 + 40 µL detection buffer	18.5 nM	3.7 nM
Control 5	20 µL Control 6 + 40 µL detection buffer	6.2 nM	1.2 nM
Control 4	20 µL Control 5 + 40 µL detection buffer	2.1 nM	0.41 nM
Control 3	20 µL Control 4 + 40 µL detection buffer	0.69 nM	0.14 nM
Control 2	20 µL Control 3 + 40 µL detection buffer	0.23 nM	0.046 nM
Control 1	20 µL Control 2 + 40 µL detection buffer	0.076 nM	0.015 nM
Control 0	40 µL detection buffer	0 nM	0 nM

ASSAY PROTOCOL

		Control	Samples
STEP 1		Dispense into each control well 4 µL of control	Dispense into each sample well 4 µL of compound/antibody or buffer
STEP 2		Dispense into all control & sample wells 8 µL of S1 d2 8 µL of ACE2 Eu Cryptate	
STEP 3		Seal the plate and incubate for Overnight at 2-8°C	
STEP 4		Remove the plate sealer and read on an HTRF® compatible reader.	

CONTROL PROTOCOL FOR INHIBITORY ASSAY IN 20 µL FINAL VOLUME

	Control	Inhibitor	ACE2 Eu Cryptate	S1 d2	detection buffer
Control	4 µL	-	8 µL	8 µL	-
Sample	-	4 µL	8 µL	8 µL	-
Positive control	-	-	8 µL	8 µL	4 µL
Negative control	-	-	8 µL		12 µL
Buffer control	-	-	-	-	20 µL

EXAMPLE OF PLATE MAP

	1	2	3	4	5	6
A	Buffer control: 20 µL detection buffer	Repeat Well A1	Repeat Well A1	Compound...: 4 µL compound... 8 µL S1 d2 8 µL ACE2 Eu cryptate	Repeat Well A4	Repeat Well A4
B	Negative control: 12 µL detection buffer 8 µL ACE2 Eu cryptate	Repeat Well B1	Repeat Well B1	Compound...: 4 µL compound... 8 µL S1 d2 8 µL ACE2 Eu cryptate	Repeat Well B4	Repeat Well B4
C	Positive control: 4 µL detection buffer 8 µL S1 d2 8 µL ACE2 Eu cryptate	Repeat Well C1	Repeat Well C1	Compound...: 4 µL compound... 8 µL S1 d2 8 µL ACE2 Eu cryptate	Repeat Well C4	Repeat Well C4
D	Ctrl 0 : 4 µL Control 0 8 µL S1 d2 8 µL ACE2 Eu cryptate	Repeat Well D1	Repeat Well D1	Compound...: 4 µL compound... 8 µL S1 d2 8 µL ACE2 Eu cryptate	Repeat Well D4	Repeat Well D4
E	Ctrl 1 : 4 µL Control 1 8 µL S1 d2 8 µL ACE2 Eu cryptate	Repeat Well E1	Repeat Well E1	Compound...: 4 µL compound... 8 µL S1 d2 8 µL ACE2 Eu cryptate	Repeat Well E4	Repeat Well E4
F	Ctrl 2 : 4 µL Control 2 8 µL S1 d2 8 µL ACE2 Eu cryptate	Repeat Well F1	Repeat Well F1	Compound...: 4 µL compound... 8 µL S1 d2 8 µL ACE2 Eu cryptate	Repeat Well F4	Repeat Well F4
G	Ctrl 3 : 4 µL Control 3 8 µL S1 d2 8 µL ACE2 Eu cryptate	Repeat Well G1	Repeat Well G1	Compound...: 4 µL compound... 8 µL S1 d2 8 µL ACE2 Eu cryptate	Repeat Well G4	Repeat Well G4
H	Ctrl 4 : 4 µL Control 4 8 µL S1 d2 8 µL ACE2 Eu cryptate	Repeat Well H1	Repeat Well H1			
I	Ctrl 5 : 4 µL Control 5 8 µL S1 d2 8 µL ACE2 Eu cryptate	Repeat Well I1	Repeat Well I1			
J	Ctrl 6 : 4 µL Control 6 8 µL S1 d2 8 µL ACE2 Eu cryptate	Repeat Well J1	Repeat Well J1			
K	Ctrl 7 : 4 µL Control 7 8 µL S1 d2 8 µL ACE2 Eu cryptate	Repeat Well K1	Repeat Well K1			
L	Ctrl 8 : 4 µL Control 8 8 µL S1 d2 8 µL ACE2 Eu cryptate	Repeat Well L1	Repeat Well L1			
M	Ctrl 9 : 4 µL Control 9 8 µL S1 d2 8 µL ACE2 Eu cryptate	Repeat Well M1	Repeat Well M1			

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 control deviation can then be worked out from ratio replicates.

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

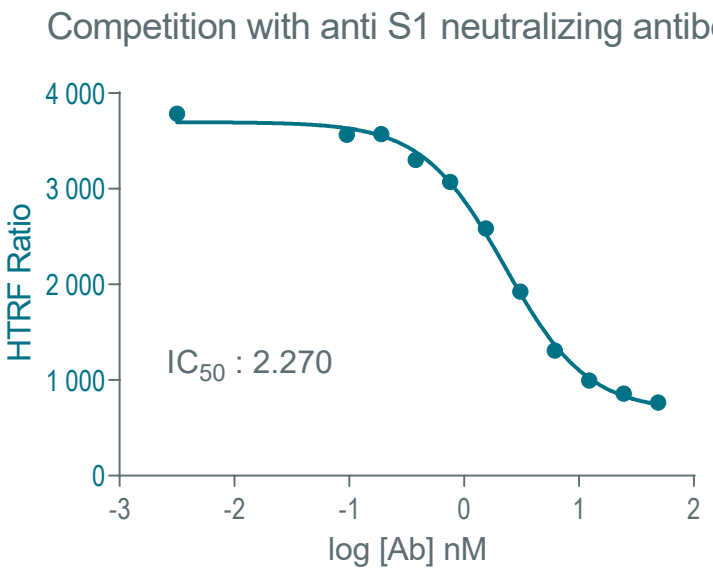
For more information about data reduction, please visit www.cisbio.com/data-reduction

RESULTS

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

Readouts on HTRF® compatible reader.

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



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