CTLA4/B7-2 BINDING ASSAY KITS

PROTOCOL

Part # 64ICP05PEG & 64ICP05PEH

Test size: 500 tests (64ICP05PEG), 10,000 tests (64ICP05PEH) - assay volume: 20 µL

Revision: 02 (July 2017)

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 CTLA4/B7-2 Binding Assay is designed to measure the interaction between CTLA4 and B7-2 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-B7-2 and Tag2-CTLA4 is detected by using anti-Tag1-Terbium (HTRF donor) and anti-Tag2-XL665 (HTRF acceptor). When the donor and acceptor antibodies are brought into close proximity due to B7-2 and CTLA4 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 CTLA4/B7-2 interaction. Thus, compound or antibody blocking CTLA4/B7-2 interaction will cause a reduction in HTRF signal.

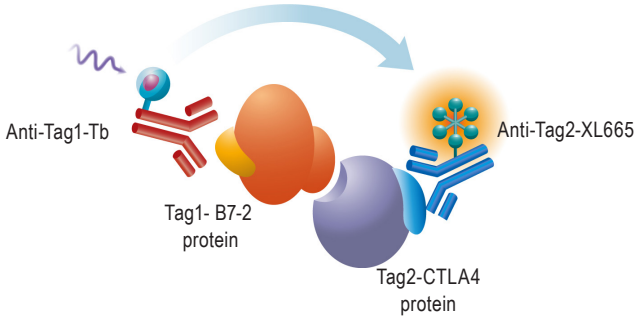
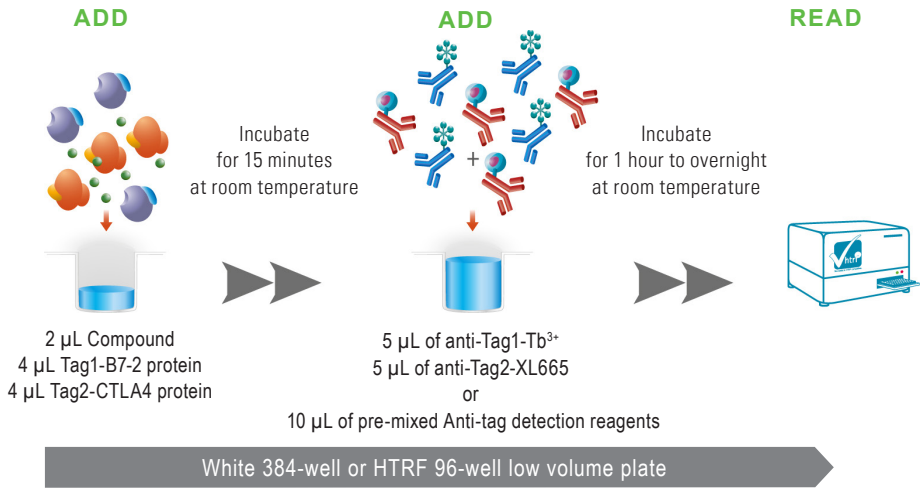


Figure 1: Principle of the HTRF CTLA4/B7-2 assay.

PROTOCOL AT A GLANCE



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

MATERIALS:

KIT COMPONENTS	500 TESTS CAT # 64ICP05PEG	10,000 TESTS CAT # 64ICP05PEH
Tag1-B7-2* MW: 26.7 kDa	1 vial Frozen see concentration and volume on vial label	1 vial Frozen see concentration and volume on vial label
Tag2-CTLA4* MW: 39.2 kDa	1 vial Frozen see concentration and volume on vial label	1 vial Frozen see concentration and volume on vial label
Anti-Tag1-Tb^{3+}	1 vial 25 µL 100 X Frozen	1 vial 0.5 mL 100 X Frozen
Anti-Tag2-XL665	1 vial 25 µL 100 X Frozen	1 vial 0.5mL 100 X Frozen
Diluent	1 vial 20 mL Cat# 62DLBDDD ready-to-use	1 vial 200 mL Cat# 62DLBDDF ready-to-use
Detection Buffer	2 vials 5 mL Cat# 62DB2FDG (130 mL) ready-to-use	1 vial 130 mL Cat# 62DB2FDG (130 mL) ready-to-use

* The amounts of Tag1-B7-2 and Tag2-CTLA4 provided are sufficient for the validated amounts of tagged proteins suitable for compound inhibition study: 6 nM of B7-2 and 6 nM of CTLA4 in 20 µL final assay volume.

For reading, an HTRF®-Certified 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 B7-2 & CTLA4 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-B7-2 protein Concentration and volume are indicated on the vial label	
Thaw the Tag1-B7-2 protein* solution. Prepare working solutions in diluent which have 5X the required final concentration for binding assay*: e.g. Prepare a 30 nM Tag1-B7-2 working solution for a final concentration of 6 nM Tag1-B7-2 (20 µL final volume).	
Tag2-CTLA4 protein Concentration and volume are indicated on the vial label	
Thaw the Tag2-CTLA4 protein* solution. Prepare working solutions in diluent which have 5X the required final concentration for binding assay*. e.g. Prepare a 30 nM Tag2-CTLA4 working solution for a final concentration of 6 nM Tag2-CTLA4 (20 µL final volume).	
Anti-Tag1-Tb^{3+}	
Thaw the anti-Tag1-Tb^{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-Tb^{3+} stock solution with detection buffer.	
e.g. 25 µL of thawed anti-Tag1-Tb^{3+} stock solution + 2475 µL of detection buffer.	e.g. 0.5 mL of thawed anti-Tag1-Tb^{3+} stock solution + 49.5 mL of detection buffer.
Anti-Tag2-XL665	
Thaw the anti-Tag2-XL665 solution. This 100 X stock solution can be frozen and stored at -60°C or below. Dilute 100-fold the 100 X anti-Tag2-XL665 stock solution with detection buffer. e.g. 25 µL of thawed anti-Tag2-XL665 stock solution + 2475 µL of detection buffer.	Thaw the anti-Tag2-XL665 solution. This allows to a 100 X stock solution, that can be frozen and stored at -60°C or below. Dilute 100-fold the 100 X anti-Tag2-XL665 stock solution with detection buffer. e.g. 0.5 mL of reconstituted anti-Tag2-XL665 stock solution + 49.5 mL of detection buffer.

*Titration of Tag1-B7-2 or Tag2-CTLA4 can be performed if necessary.

ASSAY PROTOCOL

Step 1		Dispense 2 µL of compound/antibody or diluent 4 µL of Tag1-B7-2 protein 4 µL of Tag2-CTLA4 protein.
Step 2		Incubate for 15 minutes at room temperature.
Step 3		Dispense 10 µL of pre-mixed anti-Tag1-Tb^{3+} and anti-Tag2-XL665.
Step 4		Seal the plate and incubate for 1 hour to overnight 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-B7-2	Tag2-CTLA4	Anti-Tag1-Cryptate	Anti-Tag2-XL665	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	5µL
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-B7-2 4 µL Tag2-CTLA4 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-Tb	Repeat Well B1	Repeat Well B1	**Compound...:** 2 µL compound... 4 µL Tag1-B7-2 4 µL Tag2-CTLA4 10 µL pre-mix anti-Tag reagents	Repeat Well B4	Repeat Well B4
C	**Negative control:** 6 µL diluent 4 µL Tag1-B7-2 10 µL pre-mix anti-Tag reagents	Repeat Well C1	Repeat Well C1	**Compound...:** 2 µL compound... 4 µL Tag1-B7-2 4 µL Tag2-CTLA4 10 µL pre-mix anti-Tag reagents	Repeat Well C4	Repeat Well C4
D	**Positive control:** 2 µL diluent 4 µL Tag1-B7-2 4 µL Tag2-CTLA4 10 µL pre-mix anti-Tag reagents	Repeat Well D1	Repeat Well D1	**Compound...:** 2 µL compound... 4 µL Tag1-B7-2 4 µL Tag2-CTLA4 10 µL pre-mix anti-Tag reagents	Repeat Well D4	Repeat Well D4
E	**Compound 1:** 2 µL compound 1 4 µL Tag1-B7-2 4 µL Tag2-CTLA4 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-B7-2 4 µL Tag2-CTLA4 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-B7-2 4 µL Tag2-CTLA4 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-B7-2 4 µL Tag2-CTLA4 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

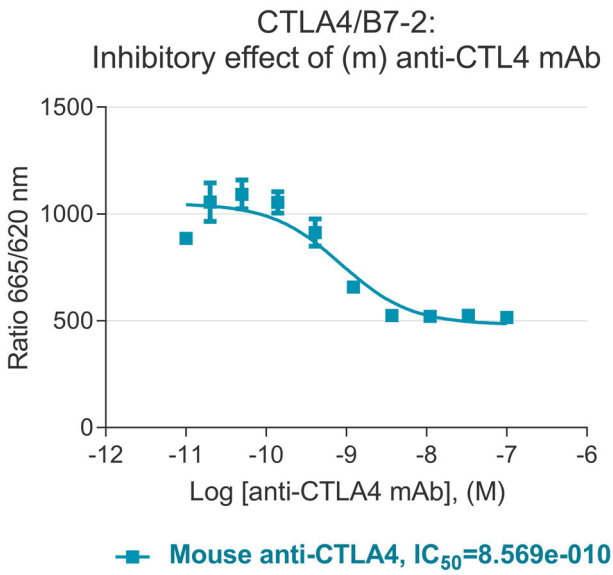
RESULTS

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 effects of mouse anti-CTLA4 were tested at 6nM CTLA4 and 6 nM B7-2.

Readouts on PHERAstar FS with a flash lamp.

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



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