

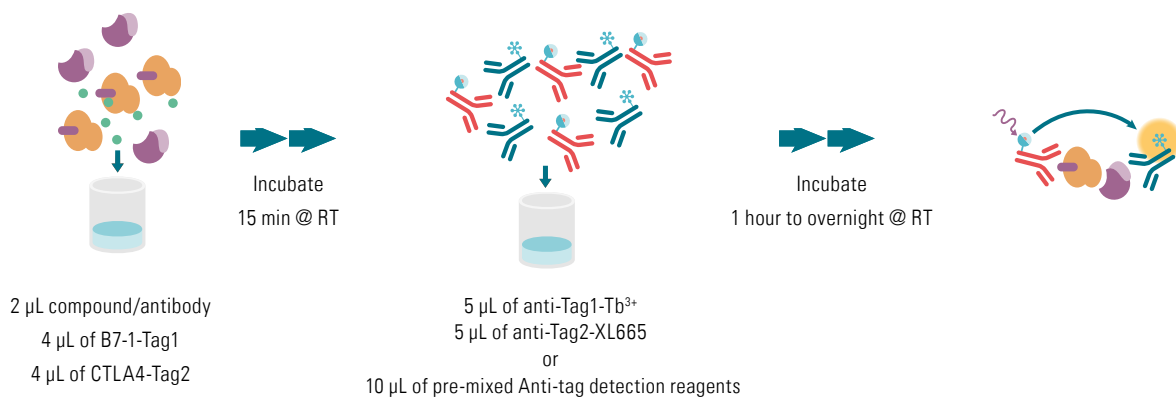


CTLA4/B7-1 BINDING ASSAY

TECHNICAL NOTE

ABSTRACT CTLA4 (cytotoxic T-lymphocyte-associated protein 4), also known as CD152, is an inhibitor of the immune response expressed by T cells. CTLA4 and CD28 (stimulatory checkpoints) share the same ligands, namely CD80 (B7-1) and CD86 (B7-2), with CTLA4 displaying a greater affinity than CD28 for both thus creating effective ligand binding competition. While CTLA4 can be found on all T cells, it is mainly expressed on Treg where it participates in their inhibition of the immune response. The HTRF CTLA4/B7-1 Binding Assay is designed to measure the interaction between CTLA4 and B7-1 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.

INHIBITION TEST



Reagents should be dispensed in the following order:

- 2 µL compound/antibody or diluent buffer.
- 4 µL B7-1-Tag1.
- 4 µL CTLA4-Tag2.

Incubate at RT for 15min.

- 5 µL of anti-Tag1-Tb³⁺ and 5 µL of anti-Tag2-XL665 or 10 µL of pre-mixture of two conjugates.

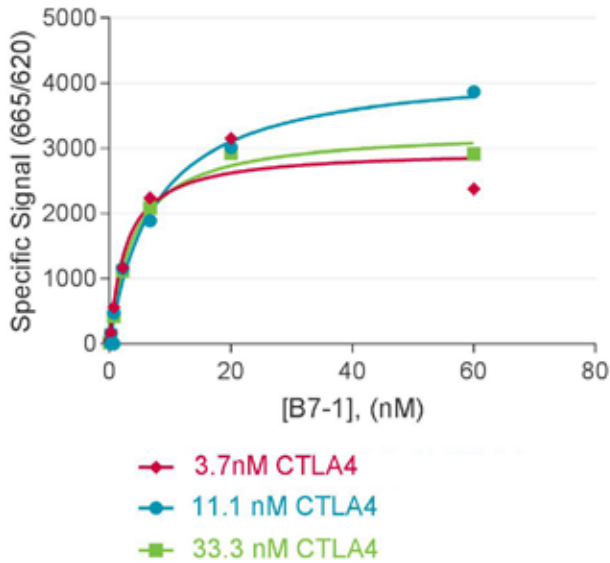
Seal the plate and incubate at RT for 1 hour to overnight.

Remove the plate sealer and read the fluorescence emission.

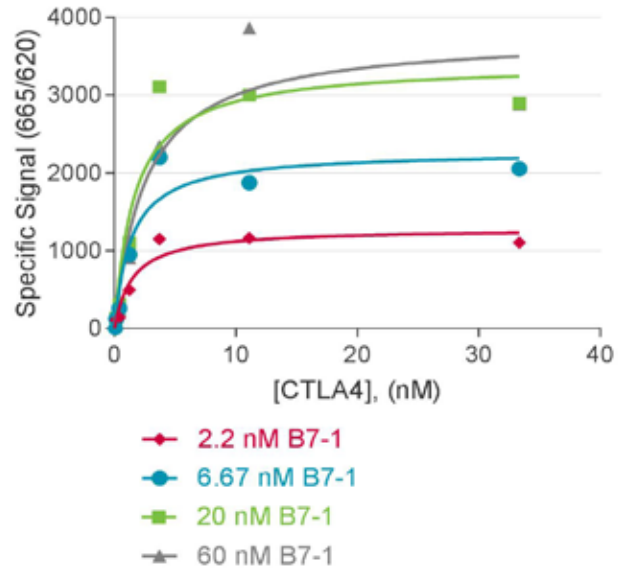
	Inhibitor	Tag1-B7-1	Tag2-CTLA4	Anti-Tag1-Cryptate	Anti-Tag2-XL665	Diluent	Detection buffer
Sample	2 µL	4 µL	4 µL	5 µL	5 µL		
Positive control		4 µL	4 µL	5 µL	5 µL	2 µL	
Negative control			4 µL	5 µL	5 µL	6 µL	
Cryptate control				5 µL		10 µL	5 µL
Buffer control						10 µL	10 µL

For 96 & 384-well low volume plates (20 µL).

CTLA4/B7-1 binding assay



CTLA4/B7-1 binding assay



In order to optimize protein concentrations, the 2 tagged proteins were cross-titrated. B7-1 was titrated from 0 to 60 nM at 3.7, 11.1 and 33.3 nM of CTLA4 and CTLA4 titration, ranging from 0 to 33.3 nM, was achieved at 2.2, 6.67, 20 and 60 nM of B7-1. The two proteins were incubated for 1 hour at room temperature.

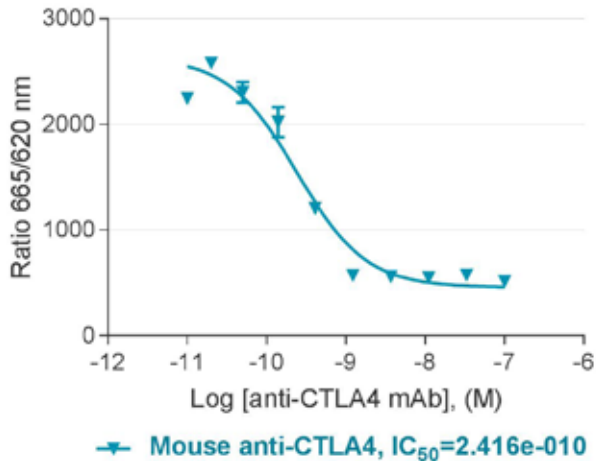
Data were calculated by fitting specific signal to One-site specific binding with hill slope.

Specific signal for each ligand concentration = Total binding - Non specific binding

3 nM CTLA4 and 3 nM B7-1 were chosen for inhibition tests.

INHIBITION OF CTLA4/B7-1

CTLA4/B7-1:
Inhibitory effect of (m) anti-CTLA4 mAb



The inhibitory effects of Mouse anti-CTLA4 were tested at 3nM CTLA4 and 3 nM B7-1. An assay window around 6 was obtained.