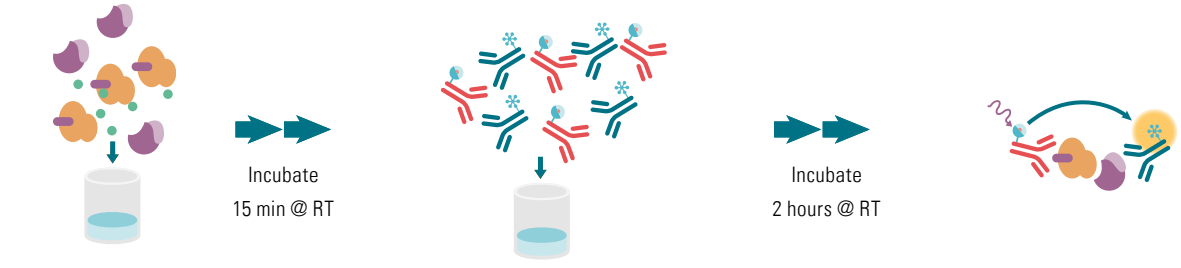
PD1/PD-L1 BINDING ASSAY

TECHNICAL NOTE

ABSTRACT Programmed cell death protein 1 (PD1) is an immune checkpoint receptor that regulates T cell response. Its ligand, programmed death-ligand 1 (PD-L1), is commonly over-expressed on the tumor cell surface. When PD1 is bound to PD-L1, T cell response is suppressed, contributing to tumor immune resistance. Checkpoint inhibitors blocking PD1/PD-L1 complex formation are generating considerable interest in cancer immunotherapy.
The HTRF PD1/PD-L1 Binding Assay is designed to measure the interaction between PD1 and PD-L1 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



2 µL compound/antibody
4 µL of PD-L1-Tag1
4 µL of PD1-Tag2

5 µL of anti-Tag1-Eu^{3+}
5 µL of anti-Tag2-XL665
or
10 µL of pre-mixed Anti-tag detection reagents

Reagents should be dispensed in the following order:

- 2 µL compound/antibody or diluent buffer.
- 4 µL PD-L1-Tag1.
- 4 µL PD1-Tag2.

Incubate at RT for 15min.

- 5 µL of anti-Tag1-Eu^{3+} and 5 µL of anti-Tag2-XL665 or 10 µL of pre-mixture of two conjugates.

Seal the plate and incubate at RT for 2 hours.

Remove the plate sealer and read the fluorescence emission.

	Inhibitor	Tag1-PD-L1	Tag2-PD1	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).

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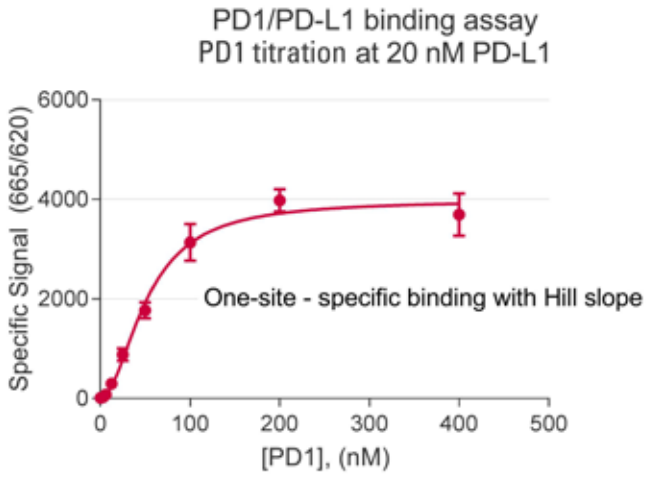
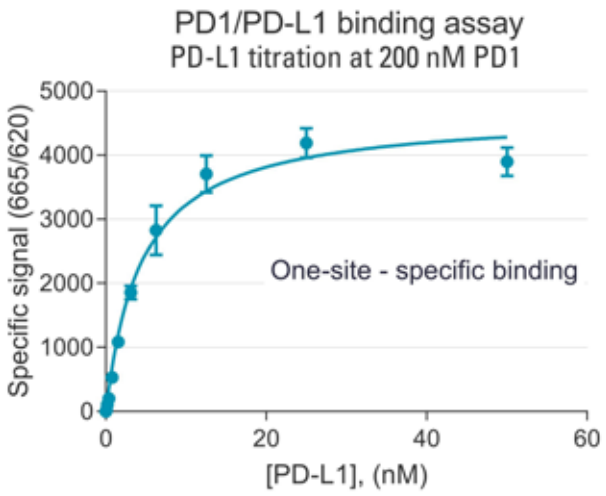
PD1/PD-L1 BINDING ASSAY





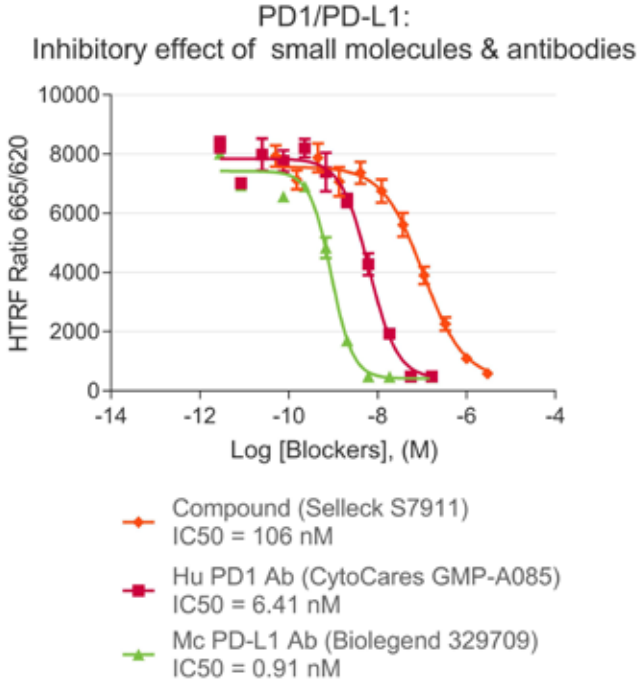
In order to optimize protein concentrations, PD1 was titrated from 0 to 400 nM at a saturating concentration of PD-L1 (20 nM) and PD-L1 was titrated from 0 to 50 nM with a saturating concentration of PD1 (200 nM). The two proteins were incubated for 2 hours at room temperature.

Data were calculated by fitting specific signal to One-site specific binding curve.

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

Optimal concentrations of PD1 and PD-L1 were defined at 50 nM and 5 nM respectively for inhibition tests.

INHIBITION OF PD1/PD-L1



The inhibitory effects of small molecules, human and mouse blocking antibodies of PD1 and PD-L1 were tested at 5 nM PD-L1 and 50 nM PD1. An assay window around 10 was obtained.

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