



6044 Cornerstone Court West, Ste E  
San Diego, CA92121  
Tel: 858-202-1401  
858-829-3082  
Fax: 858-481-8694  
Email: [Info@bpsbioscience.com](mailto:Info@bpsbioscience.com)

# Sample Assay Report

## Immunotherapy Inhibitor Assays Sample Report



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Fax: 858-481-8694  
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## Immunotherapy Inhibitor Assays

Study Sponsor:

Attention:

Address:

Study Director: Henry Zhu, Ph.D.

Testing Facility: BPS Bioscience Inc.  
6042 Cornerstone Court West, Ste. B  
San Diego, CA 92121  
USA

Study Period:

Report Version: 1

Report Date:



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## Study Director

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Aaron Snead  
Senior Scientist II, Ph.D.

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Date

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Henry Zhu, Ph.D.  
President

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Date

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## **1. Purpose of the Study**

The purpose of the study is to determine the effect of test compounds from Sample Report on the activity of various immunotherapy targets.

## 2. Materials and Methods

### 2.1 Materials

Protein	Catalog #	Lot #
PD-1	71106	131113-D
PD-L1-biotin	71105	150312
PD-1 mAb	71120	140213-D
PD-L2-biotin	71108	130918
PD-L1	71104	150127-D
B7-1-biotin	71114	150128-B
PD-L1 mAb	71213	140716-D
BTLA	71141	140408-D
HVEM-biotin	71143	140417-BD
HVEM	71142	140422
CD28	71113	140109
CTLA4	71149	140414
CTLA4 Ab	71212	140708-D
CD47	71177	140429
SIRP $\alpha$ -biotin	71138	140402
SIRP $\alpha$	71145	140331
OX40L	71185	140708-D
OX40-biotin	71310	150722
OX40	71175	150616

### 2.2 Compounds

Compound I.D.	Compound Supplied	Stock Concentration	Dissolving Solvent	Test Range

## 2.3 Experimental Conditions

### 2.3.1 Proteins and Substrates

Protein	Catalog #	Lot #	Protein Used /reaction
PD-1	71106	131113-D	100 ng
PD-L1-biotin	71105	150312	40 ng
PD-1 mAb	71120	140213-D	30 nM ref
PD-L2-biotin	71108	130918	20 ng
PD-L1	71104	150127-D	250 ng
B7-1-biotin	71114	150128-B	25-150 ng
PD-L1 mAb	71213	140716-D	30 nM ref
BTLA	71141	140408-D	100 ng
HVEM-biotin	71143	140417-BD	100 ng
HVEM	71142	140422	1 $\mu$ M ref
CD28	71113	140109	100 ng
CTLA4	71149	140414	100 ng coat (1 $\mu$ M ref)
CTLA4 Ab	71212	140708-D	30 nM ref
CD47	71177	140429	100 ng
SIRP $\alpha$ -biotin	71138	140402	600 ng
SIRP $\alpha$	71145	140331	3 $\mu$ M ref
OX40L	71185	140708-D	200 ng
OX40-biotin	71310	150722	20 ng
OX40	71175	150616	500 nM ref

### 2.3.2 Assay Conditions

Experimental conditions were done according to the Respective Binding Assay Kit Protocol (<http://bpsbioscience.com/immunotherapy/assay-kits>). Coat proteins were added to the plate 50  $\mu$ L at 2-5 ng/ $\mu$ L at 4  $^{\circ}$ C overnight. Test compounds or reference compound/neutralizing antibody were added to the coated plate followed by addition of the corresponding biotinylated binding partner. Reaction was incubated for 2 h at room temperature.

### 2.3.3 Data Analysis

Binding assays were performed in duplicate at each concentration. The luminescence data were analyzed using the computer software, Graphpad Prism. Percent inhibition was determined by normalizing the data to signal from Negative Control wells (uncoated wells treated with the biotinylated ligand, set as 100% inhibition) and Positive Control wells (coated wells treated with the biotinylated ligand in the absence of any inhibitor, set as 0% inhibition). Data for a reference compounds or antibodies are included as a control for inhibition.





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### **3. Assay Results**

#### **3.1. Summary of the Inhibitory Effects of the Compounds on Immunotherapy Inhibitory Assays**

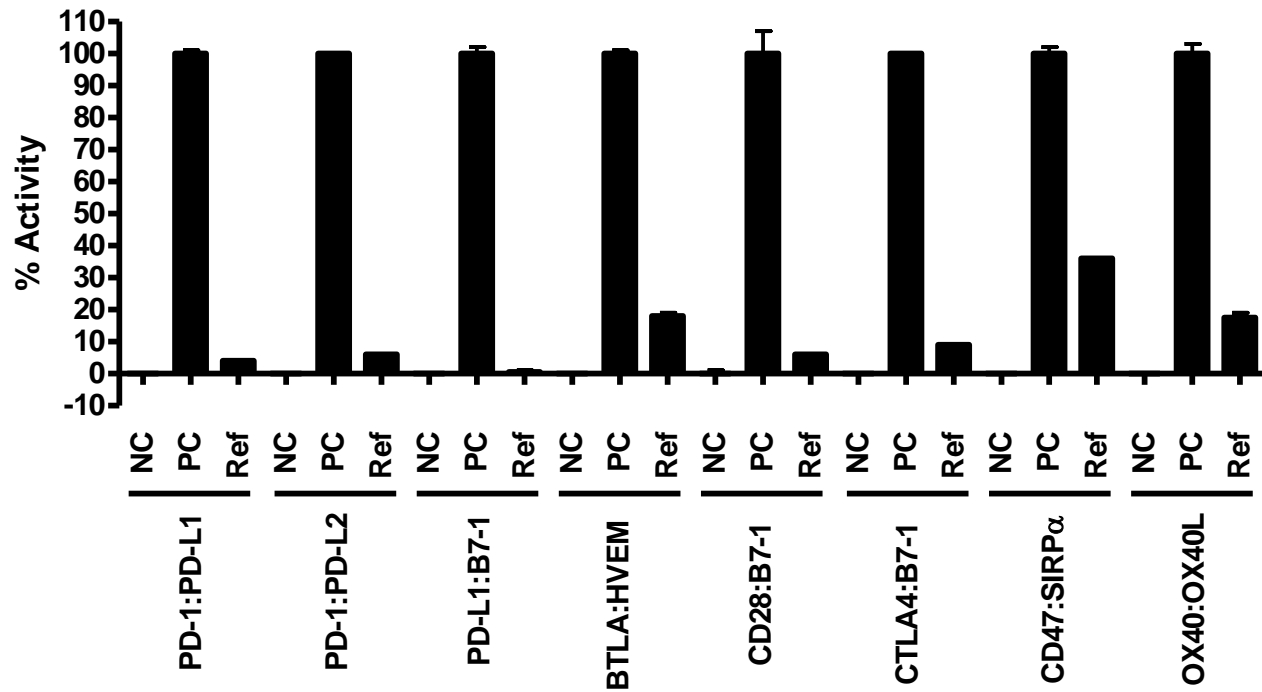
### 3.2. Results of the Effects of the Compounds on Immunotherapy Assays

#### 3.2.1 Results of the Effects of the Compounds on Immunotherapy Assays

Table 3.2.1.

Activity of Compounds						
Assay	Condition	Concentration	Raw Data		% Activity	
			Repeat 1	Repeat 2	Repeat 1	Repeat 2
PD-1:PD-L1	Negative Control		281	278	0	0
	Positive Control		32811	32081	101	99
	Reference Inhibitor	30 nM PD-1 Ab	1451	1535	4	4
PD-1:PD-L2	Negative Control		331	342	0	0
	Positive Control		51852	51508	100	100
	Reference Inhibitor	30 nM PD-1 Ab	3208	3230	6	6
PD-L1:B7-1	Negative Control		167	198	0	0
	Positive Control		21595	22539	98	102
	Reference Inhibitor	30 nM PD-L1 Ab	388	218	1	0
BTLA:HVEM	Negative Control		1731	1319	0	0
	Positive Control		56766	57777	99	101
	Reference Inhibitor	1 $\mu$ M HVEM	11045	11980	17	19
CD28:B7-1	Negative Control		2118	1422	1	-1
	Positive Control		47050	40995	107	93
	Reference Inhibitor	100 nM CTLA4	4289	4118	6	6
CTLA4:B7-1	Negative Control		30	35	0	0
	Positive Control		54427	54584	100	100
	Reference Inhibitor	30 nM CTLA4 Ab	4742	4933	9	9
CD47:SIRP $\alpha$	Negative Control		1437	1295	0	0
	Positive Control		57327	55224	102	98
	Reference Inhibitor	30 $\mu$ M SIRP $\alpha$	21122	20896	36	36
OX40:OX40L	Negative Control		803	743	0	0
	Positive Control		33567	31956	103	97
	Reference Inhibitor	5 $\mu$ M OX40	6831	5981	19	16

### Sample Immunotherapy Binding Data





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## **Storage and Retention of Records**

The original final report provided to the sponsor will be kept by the sponsor under its sole responsibility.



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## **5. Quality Assurance Statement**

I certify that the results presented in this report were generated using the materials and methods mentioned and that these results reflect the Raw Data.

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Henry Zhu, Ph.D.  
President

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Date