



PathHunter[®] PD-1 Signaling Bioassay

Qualified with Keytruda[®]

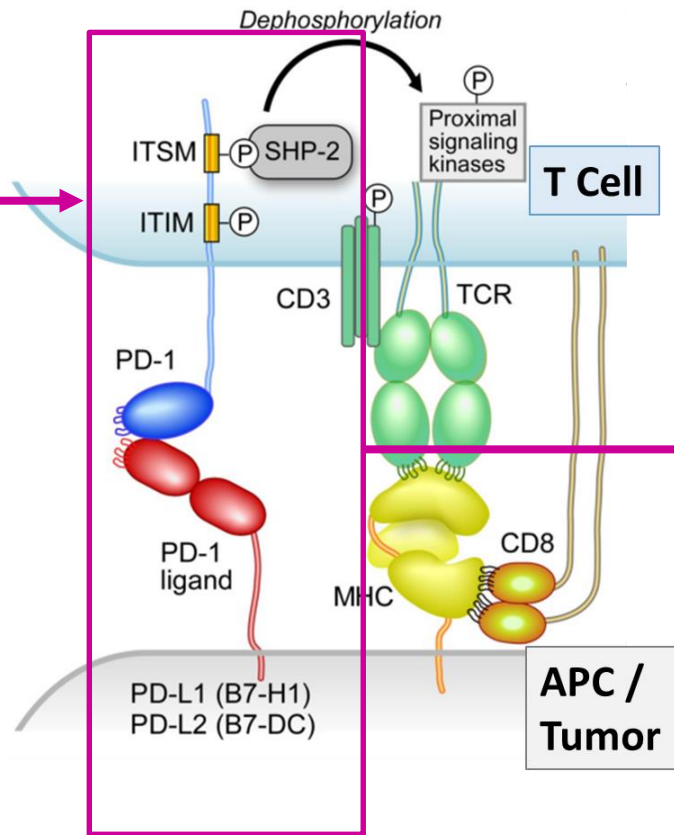
[93-1104Y19-00117](#) (2-Plate Kit)

[93-1104Y19-00118](#) (10-Plate Kit)

OUR EXPERTISE
IN YOUR HANDS.
DISCOVER
CONFIDENTLY.

PD-1 Signaling Assay: Measure early step in PD-1 mediated inhibition of T cell activation

Mechanism of Action



Assay measures this early event: SHP recruitment

Assay Design

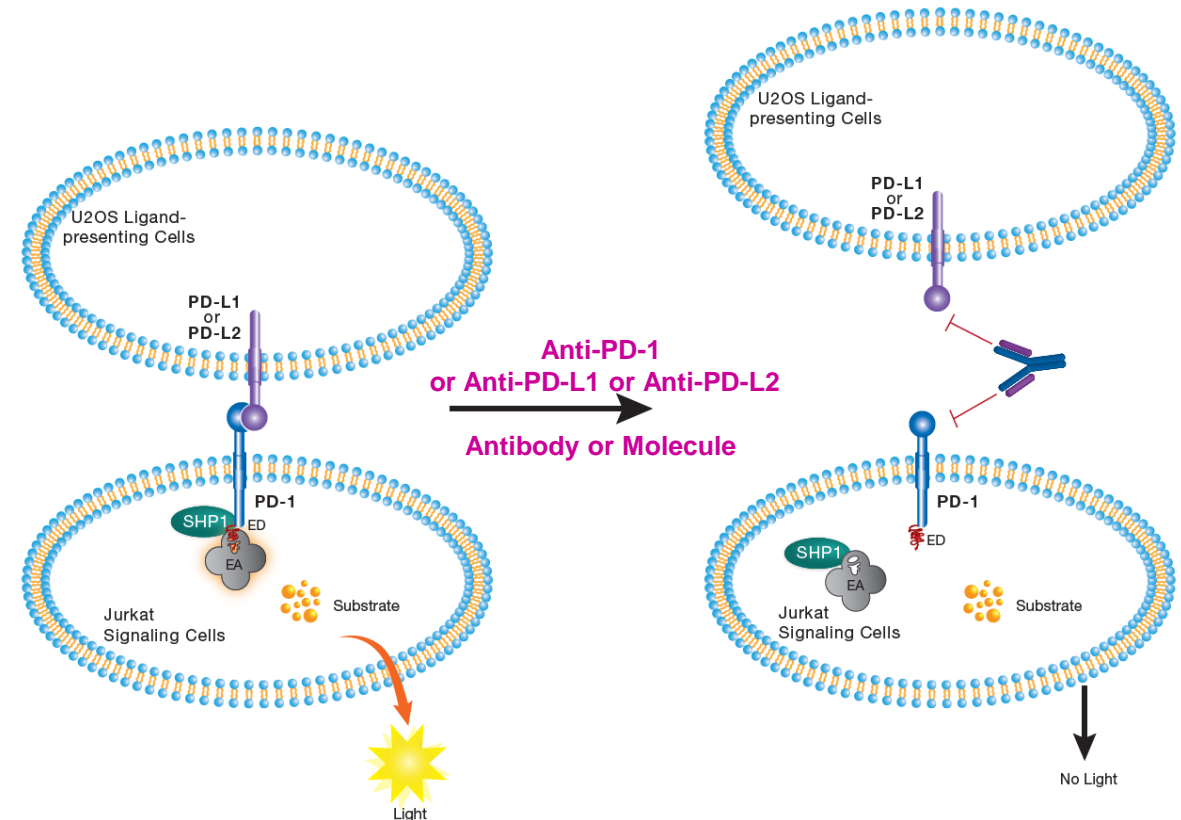


Figure from Science Webinar Series, Part 5: Gordon J. Freeman, Ph.D.

PathHunter® PD-1 Signaling Bioassay Kit

Kit Components

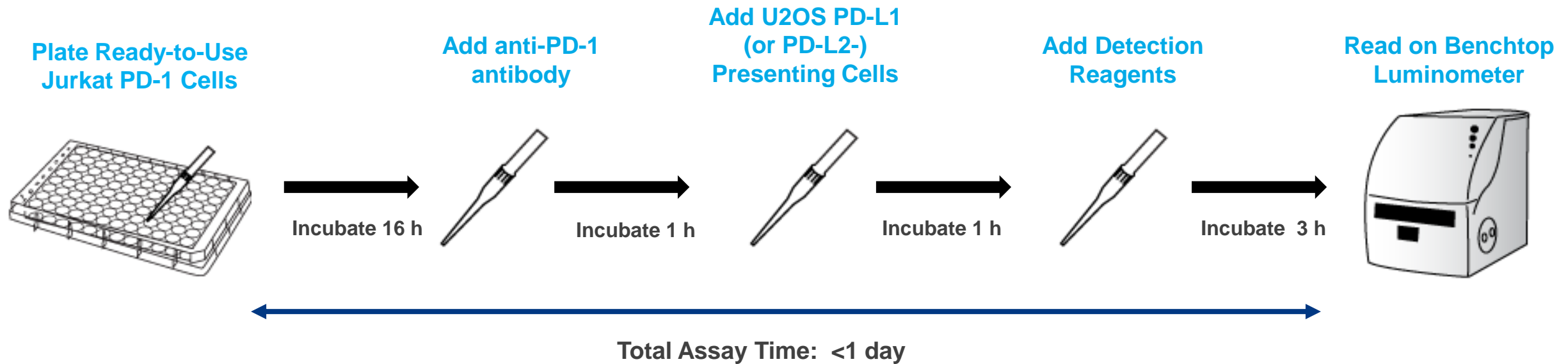
List of Components	93-1104Y19-00117	93-1104Y19-00118
PathHunter Jurkat PD-1 Bioassay Cells	2 vials	10 vials
PathHunter U2OS PD-L1 Bioassay Cells	2 vials	10 vials
PathHunter Bioassay Detection Kit		
Detection Reagent 1 (mL)	2	10
Detection Reagent 2 (mL)	8	40
AssayComplete™ Cell Plating 0 Reagent	1 X 100 mL	2 X 100 mL
Anti-PD-1 antibody	1 vial	1 vial
96-Well White, Flat-Bottom, TC-Treated, Sterile Plates with Lid	2 plates	10 plates

Sample data

PD-1 Signaling Bioassay Kit Qualification

PD-1 Signaling Bioassay Workflow

Simple, Homogenous and Rapid Protocol



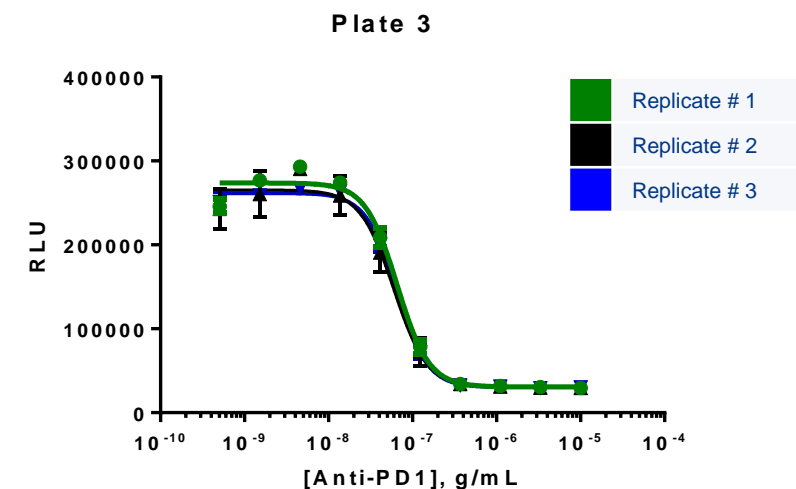
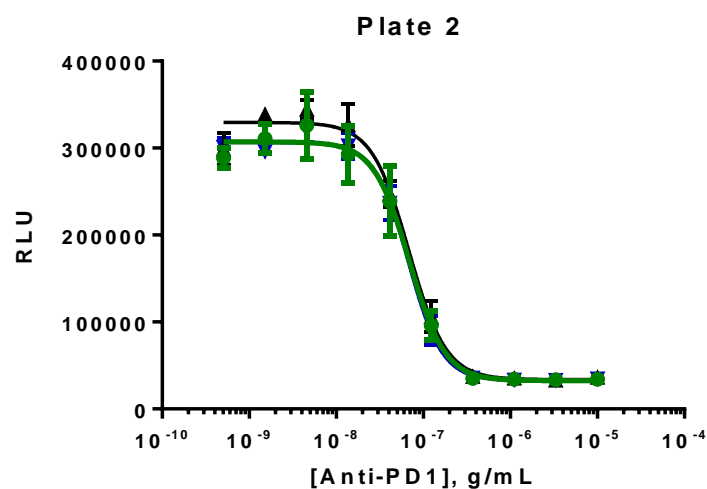
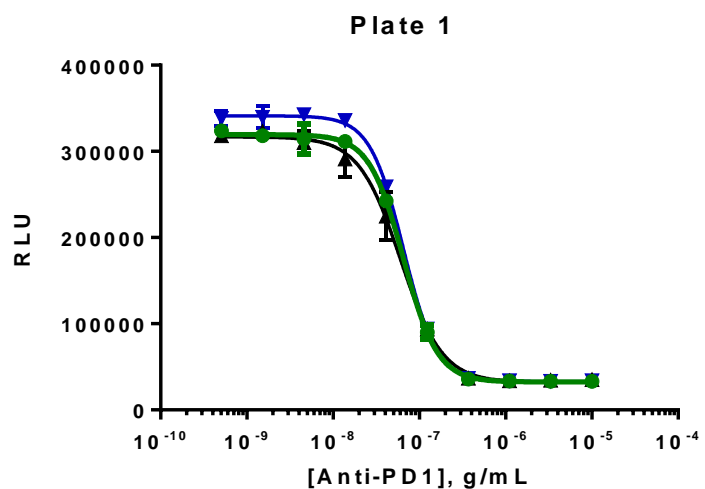
PD-1 Signaling Bioassay Qualification

Assay Parameters Assessed

- % CV between 8 full plate DRCs
- Plate-to-Plate variability: 3 plates with full plate DRCs run on 3 days
- Relative potency across range of 50-150%
- Accuracy, precision, linearity and parallelism of assay run by a single operator

PD-1 Signaling Bioassay Qualification

Plate-to-Plate Variability: 3 Plates with Full-plate DRC - Same Day (D3)



Parameter	R1	R2	R3
S/B	9.7	9.2	10.2
Hill Slope	-2.247	-1.861	-2.355
IC ₅₀ (ng/mL)	65.2	60.5	65.2

Parameter	R1	R2	R3
S/B	9.5	9.5	9.3
Hill Slope	-2.239	-2.179	-2.398
IC ₅₀ (ng/mL)	69.1	68.4	67.2

Parameter	R1	R2	R3
S/B	9.9	9.8	8.8
Hill Slope	-2.46	-2.288	-2.49
IC ₅₀ (ng/mL)	65.5	59.8	64.5

Assay Robustness

Repeatability and Intermediate Precision (Inter-Plate)

Plate	Sample	S/B	%RSD, S/B	IC ₅₀ , ng/mL	Mean IC ₅₀ , ng/mL	%RSD, IC ₅₀
1	R1	9.7	5.2	65.2	63.6	4.26
	R2	9.2		60.5		
	R3	10.2		65.2		
2	R1	9.5	1.2	69.1	68.2	1.4
	R2	9.5		68.4		
	R3	9.3		67.2		
3	R1	9.9	6.4	65.5	63.2	4.8
	R2	9.8		59.8		
	R3	8.8		64.5		

Intermediate Precision (Inter-Day)

Day	IC ₅₀ , ng/mL	Mean IC ₅₀ , ng/mL	%RSD, IC ₅₀
1	56.3	64.6	12.5
2	72.4		
3	65		

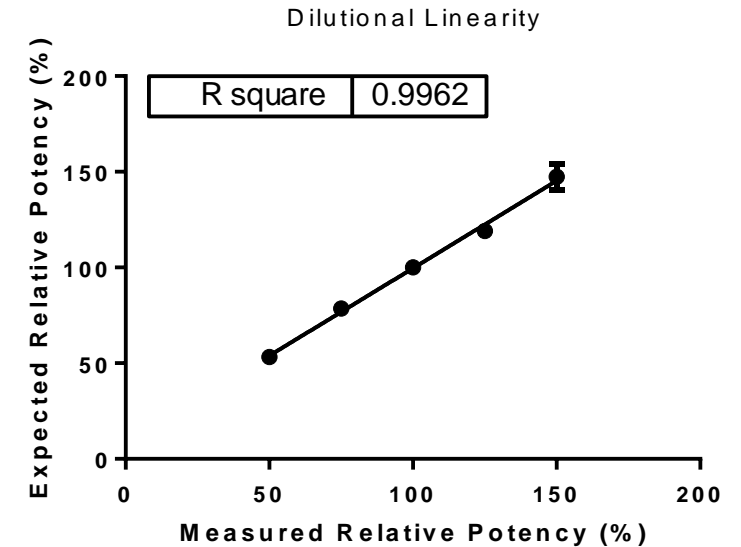
Intermediate Precision (Inter-Plate): 4.8%
Intermediate Precision (Inter-Day): 12.5%

PD-1 Signaling Bioassay: Qualification with Keytruda®

Example of Accuracy, Precision and Dilutional Linearity: Single Analyst

Exp #	Expected RP (%)	Observed RP (%)	Average RP (%)	% RSD	% Recovery
1	150	142	147	4.6	98.2
2		155			
3		145			
1	125	119	119	1.7	95.2
2		117			
3		121			
1	75	78	79	0.7	104.8
2		78.9			
3		78.9			
1	50	54.9	53	5.9	106.6
2		55.3			
3		49.7			

Note: RP values calculated in GraphPad Prism

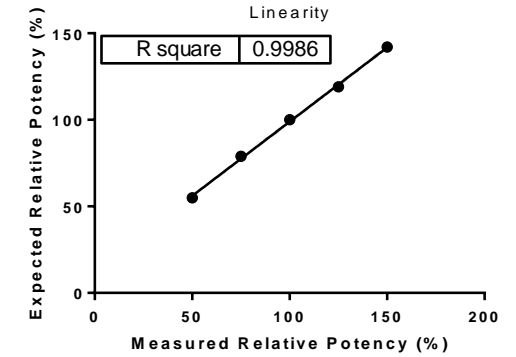
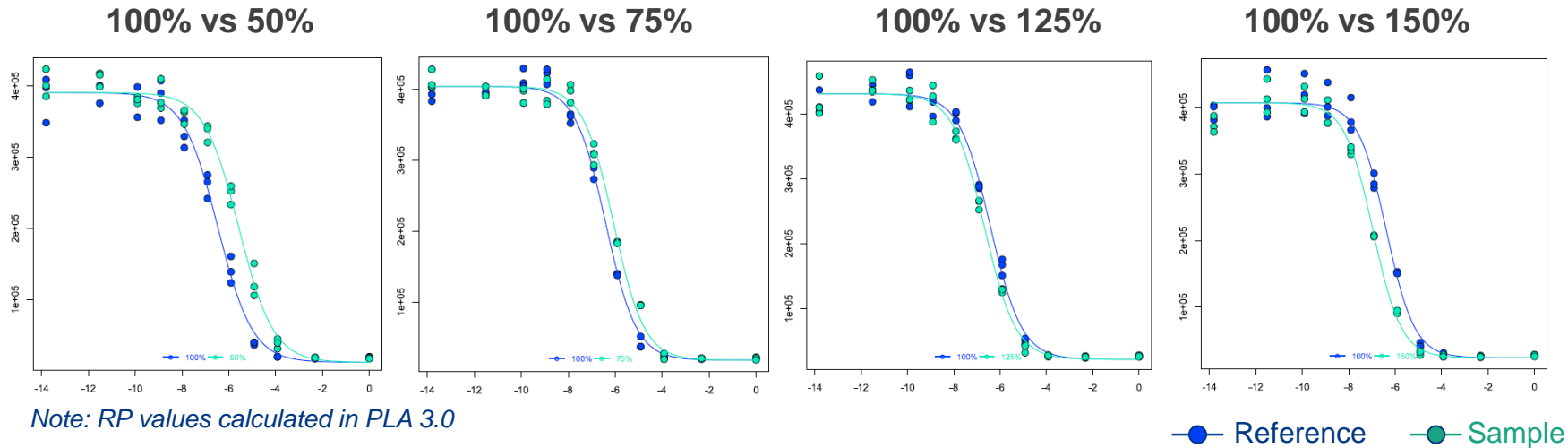


Accuracy: 101.2%

Precision: 3.1%

PD-1 Signaling Bioassay: Qualification with Anti-PD-1 therapeutics

Relative Potency: Pembrolizumab (Keytruda®) & Nivolumab (Opdivo®)



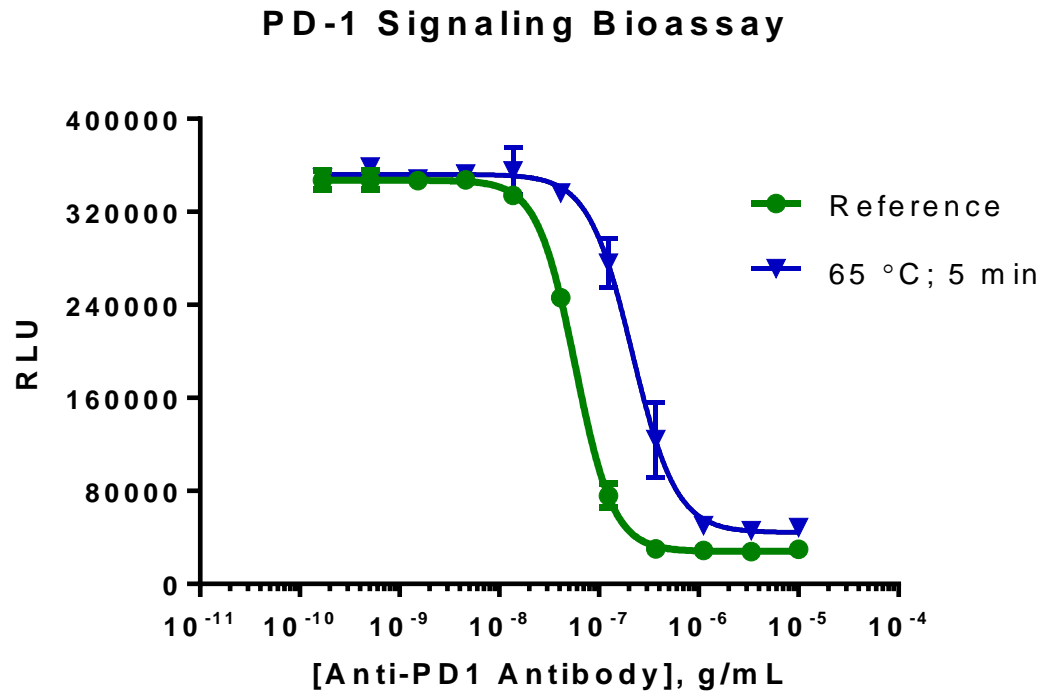
Relative Potency Data for Pembrolizumab

Drug	Accuracy	Precision	Linearity
Pembrolizumab	99.7%	4.8%	0.994
Nivolumab	100.5%	6.0%	0.998

Opdivo® is a registered trademark of Bristol-Myers Squibb Company

PD-1 Signaling Bioassay: Stability-Indicating

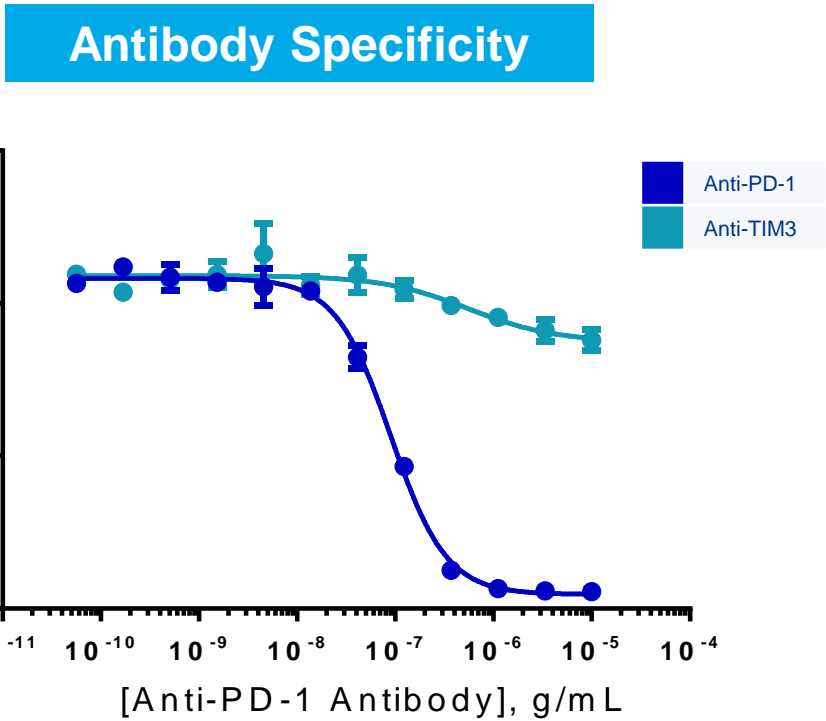
Clearly differentiates heat-stressed material from reference sample



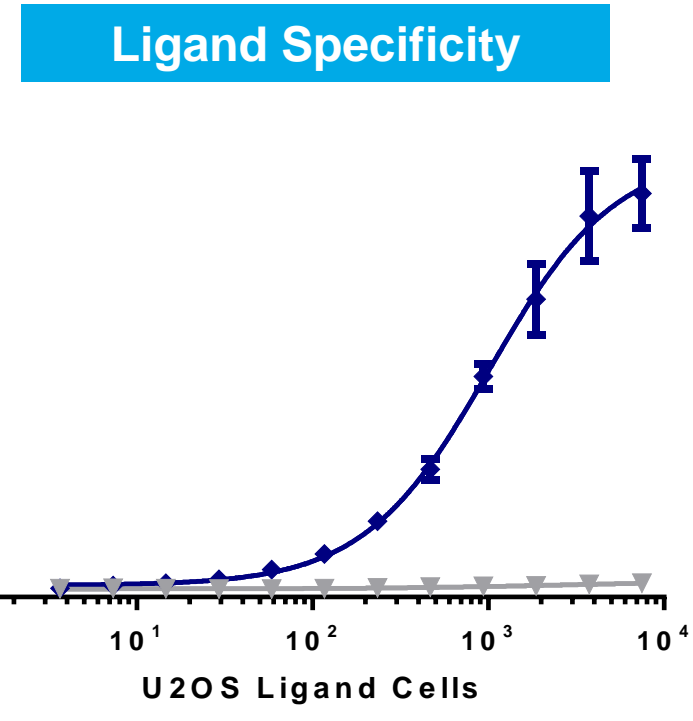
Parameter	Reference	65 °C; 5 min
S/B	11.7	7.4
Hill Slope	-2.282	-1.978
IC ₅₀ (ng/mL)	57.6	21.53

PD-1 Signaling Bioassay: Specificity

Demonstrates antibody and ligand specificity



Antibody	S/B	Hill Slope	IC ₅₀ (ng/mL)
Anti-PD-1	20.2	-1.532	89.95
Anti-TIM3	1.3	-0.975	556.7



	S/B
PD-1 + U2OS HVEM	--
PD-1 + U2OS PDL2	46

Benefits for “Ready-to-Use” Bioassay Kits

Functional response based on drug MOA

Simple protocol; Rapid results

Specific and Sensitive assay

Highly reproducible

Readily Implement with Optimized kit

- Frozen ready-to-assay cells
- Bioassay Detection Reagents
- Cell Plating Reagent
- Dilution Buffer
- Control Agonist
- Tissue Culture-Treated Plates

For More Info, Questions or Technical Support



Web:

[Cell-Based Bioassays for Biologics](#)

Technical Support

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