



Qualification of Ready-to-Use Cell-Based Assays for Potency to Support Immunology and Immunotherapy Drug Development

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BEBPA Virtual Conference

OUR EXPERTISE
IN YOUR HANDS.
DISCOVER
CONFIDENTLY.

Eurofins DiscoverX

Strong Foundation | Technical Expertise | End-to-End Solutions



Dedicated Operations

Supporting programs from Research, Discovery to Lot Release

- **Products Division headquartered in Fremont, CA**
- Additional sites in Missouri, USA and Poitiers, France
- **800+ off-the-shelf assays for in-house development**
- Over 10,000 customers in NA, APAC and EMEA

Deep Domain Expertise

Over 45 years of cumulative technical experience in

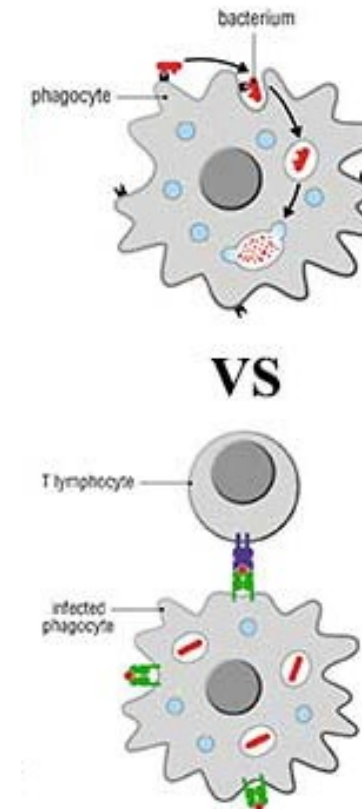
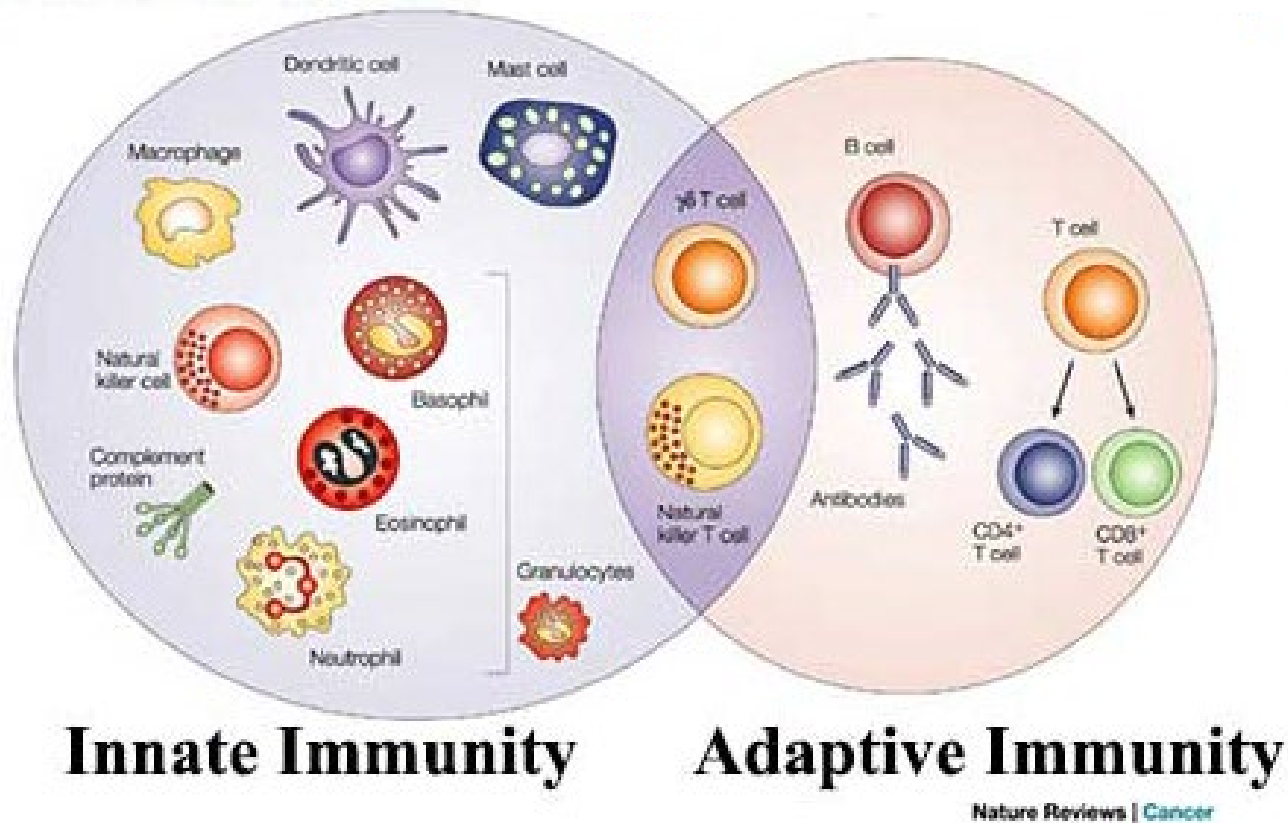
- **Cell line engineering & characterization**
- **Bioassay development, optimization & qualification**
- Analytical Cell Banks
- Membrane Preps and Frozen Assay Ready Cells
- Bulk Enzyme Production

Established Brand

Successfully implemented at global Pharma, Biotech & CRO

- **Products implemented in discovery & development**
- Over 50 billion data points screened
- **2,000+ publications**
- Several active Biotech/CRO-partnered programs
- **Implemented in lot release of several marketed biologics**

Immunotherapy Agents: Targeting Innate vs Adaptive Immunity



Therapeutic Modalities

TLR Agonists
STING Agonists
SIRP α / CD47

Checkpoint Inhibitors
(anti-PD-1/PD-L1)

Checkpoint agonists
(OX40, CD137, ICOS)

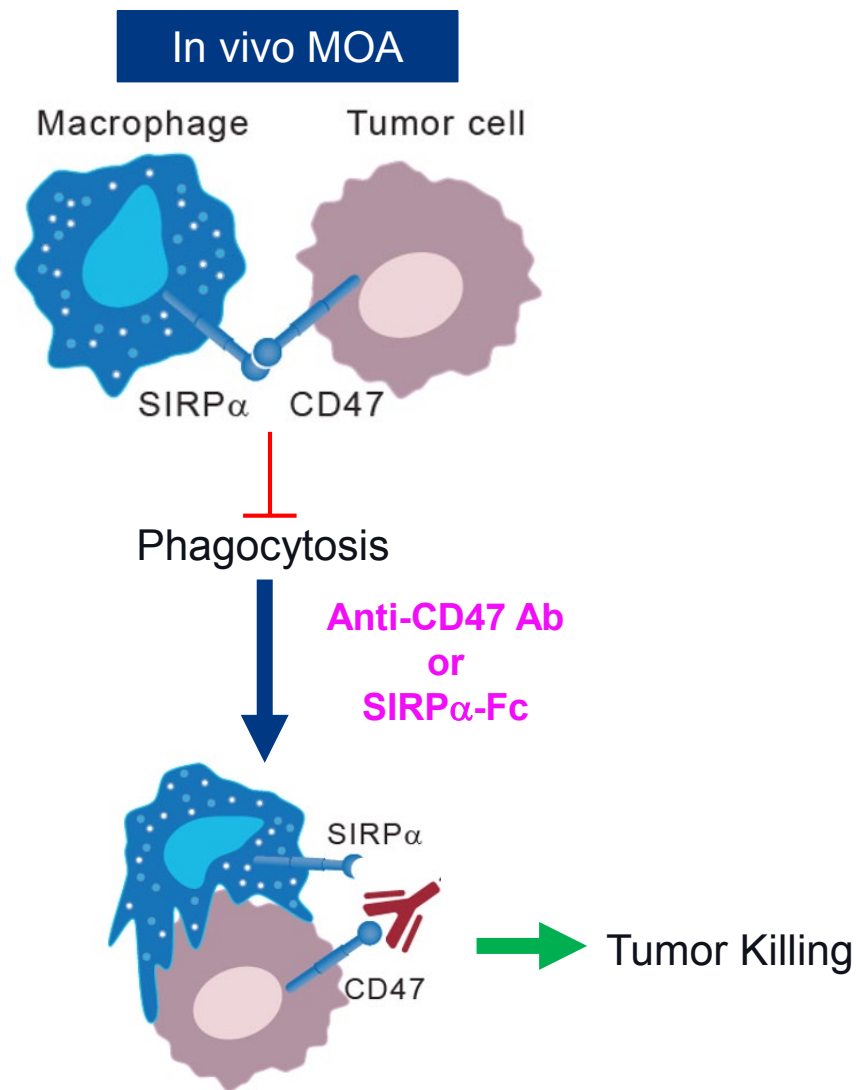
BiTEs, TRIKEs, etc

Case Study 1

Development and Qualification of an Assay-Ready SIRP α (CD47) Signaling Assay

The SIRP α / CD47 Axis

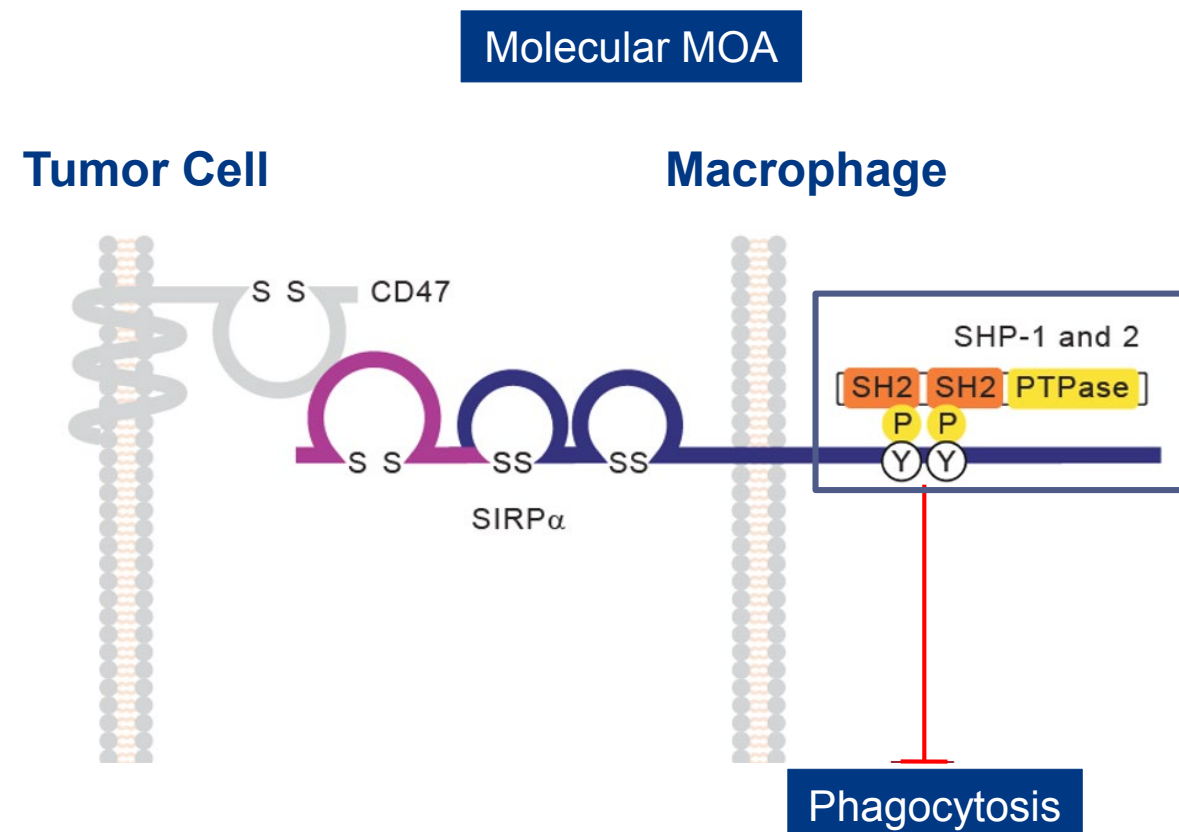
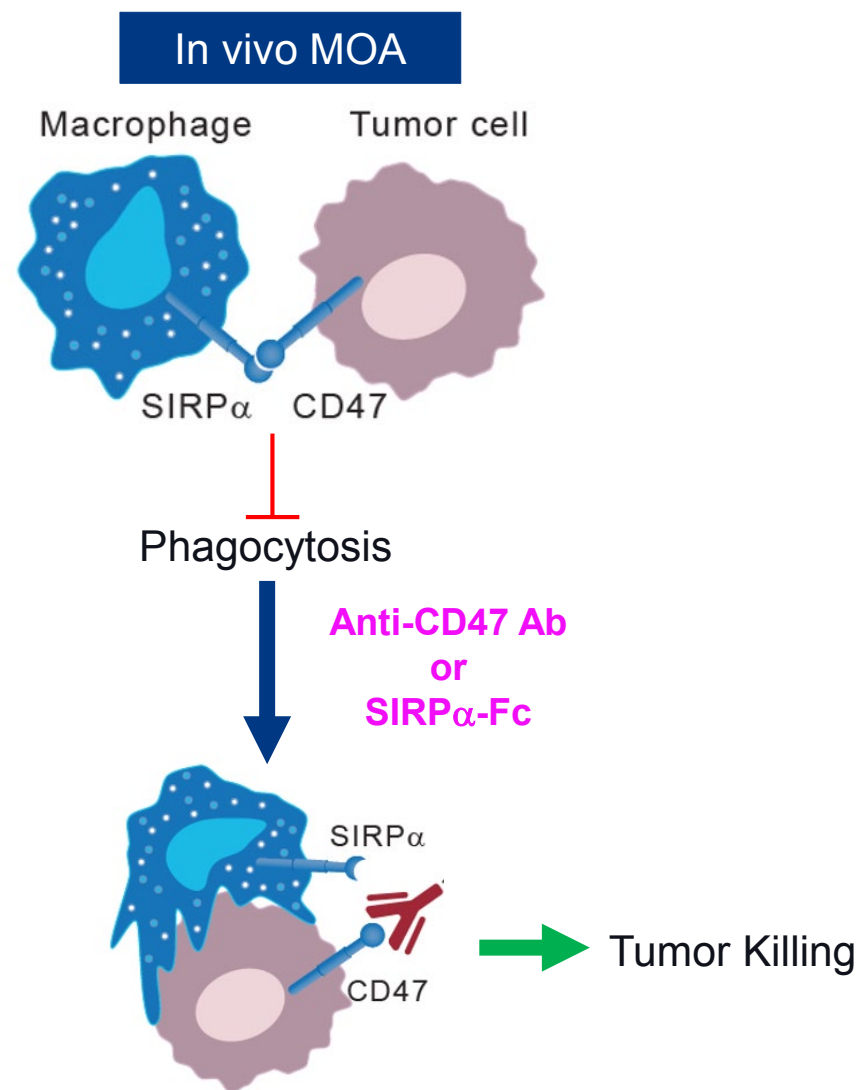
A Macrophage Immune Checkpoint



- SIRP α is a receptor expressed on macrophages and dendritic cells that promotes phagocytosis of foreign objects
- CD47, the ligand for SIRP α , is expressed on nearly all cells, but is significantly up-regulated in many tumor types, especially hematological malignancies such as AML and MDS
- ‘Don’t eat me’ signal that represses signaling via SIRP α and prevents phagocytosis
- Blocking the CD47 / SIRP α axis (e.g. with anti-CD47 antibodies, engineered receptor decoys, anti-SIRP α antibodies and bispecific agents) promotes phagocytosis of the tumor
- Anti-CD47 blockade has also been shown to enhance adaptive immunity (e.g. prime an anti-tumor cytotoxic T cell response)



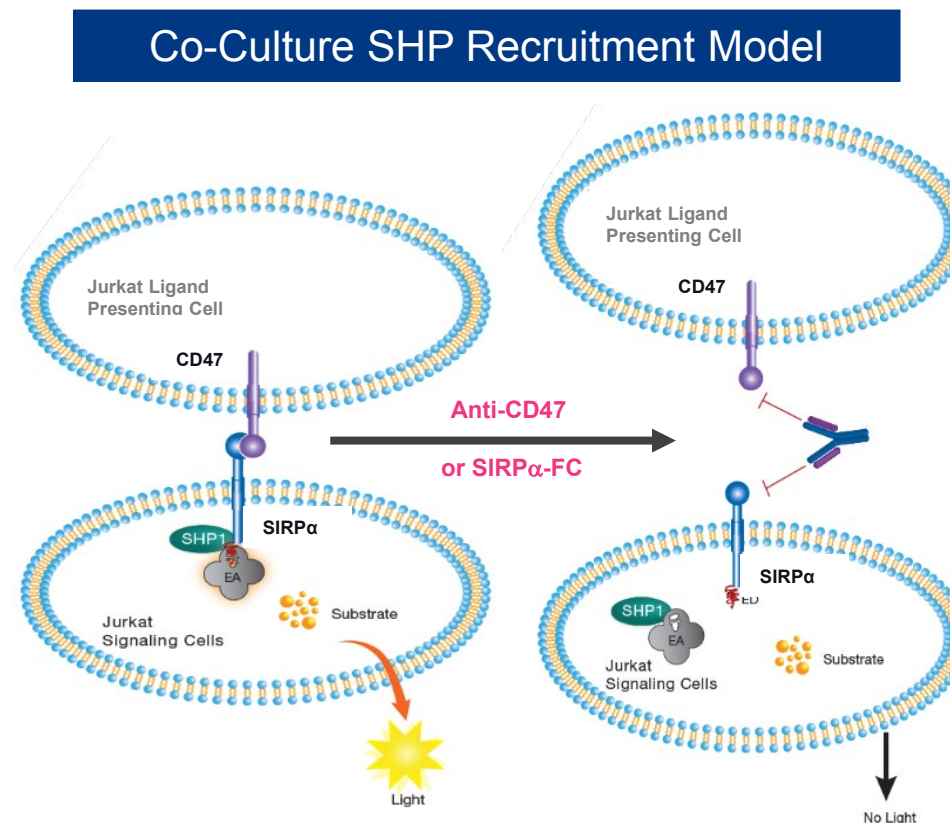
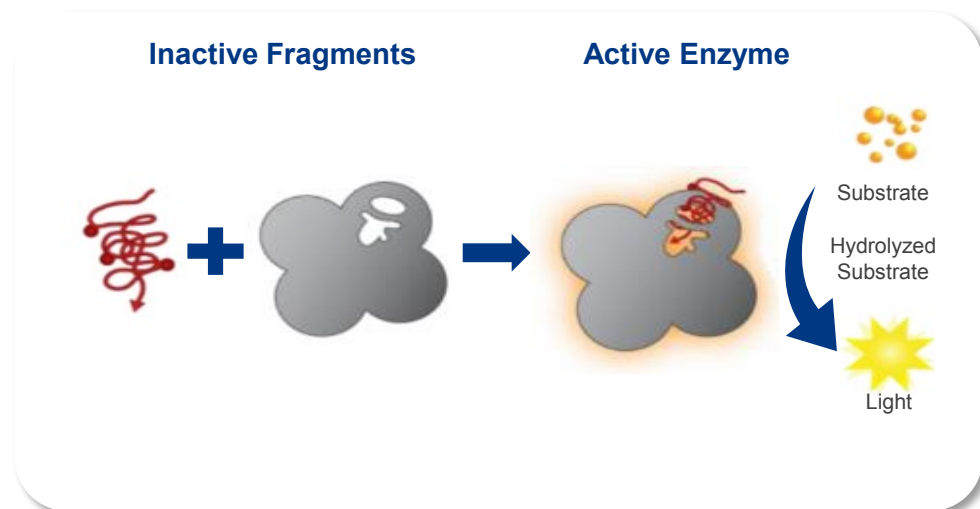
Molecular MOA of SIRP α / CD47 Signaling Axis



Adapted from Trends in Cell Biology, 2008. Vol 19, No. 2

PathHunter® SIRPα Signaling Assay: Assay Concept

Co-culture SHP recruitment model based on β -galactosidase enzyme fragment complementation



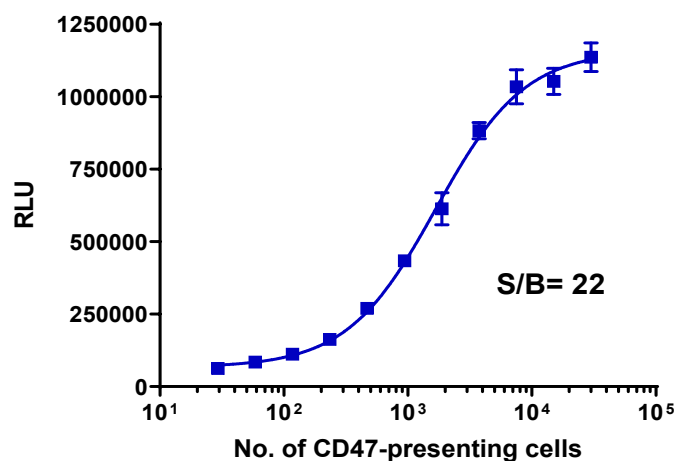
Assay quantifies ligand-induced recruitment of SHP-1 to ITIM motifs in C-terminal tail of SIRPα in response to phosphorylation

PathHunter® SIRPα (CD47) Signaling Assay

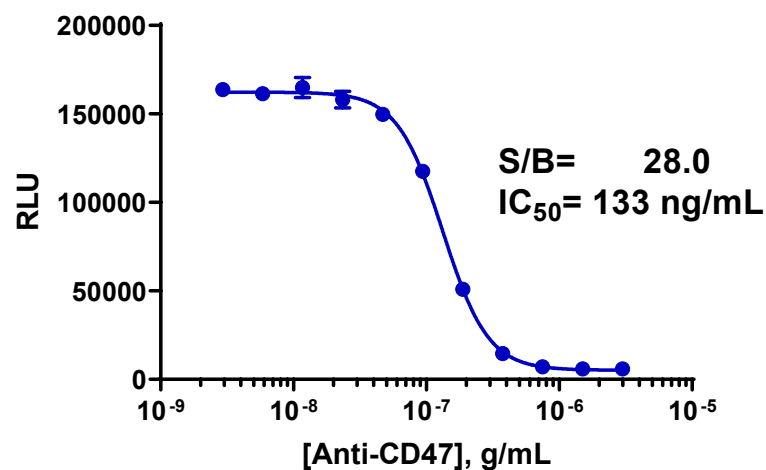
Co-culture model with stable surface expression of SIRPα and a stable functional response over 45+ passages

Cell-Presented Ligand

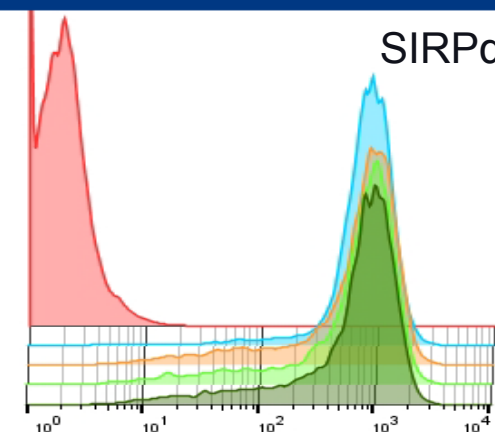
Agonist



Antagonist

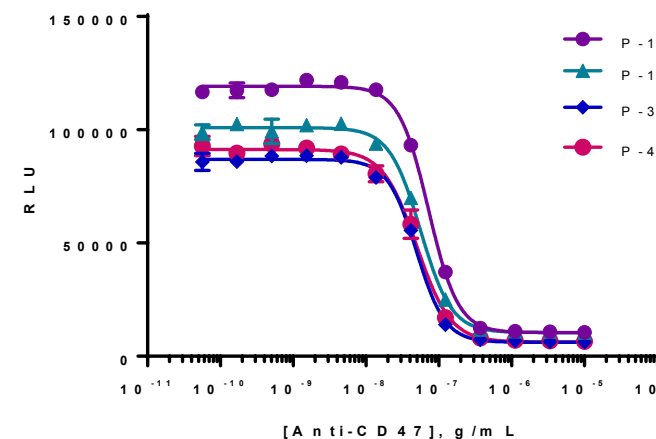


Stable SIRPα Surface Expression



SIRPα expression varies by <20% RSD over 45 passages

Stable Functional Response



S/B : 17% RSD over 45 passages

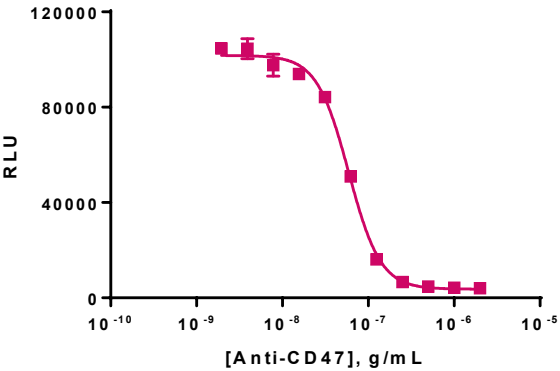
IC₅₀ : <19% RSD over 45 passages

Development of RTU Assay Format for SIRPα Signaling Assay, with an Easy-to-Transfer Method

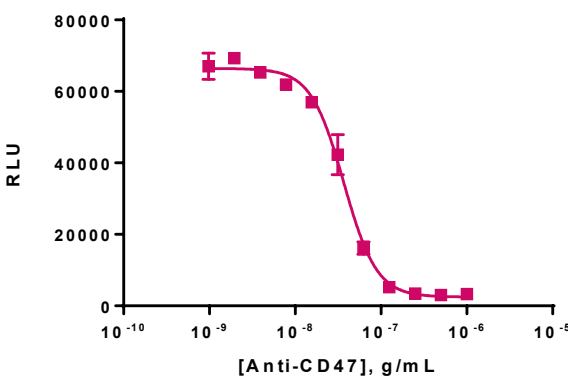
RTU (Bioassay) Method



Continuous Culture



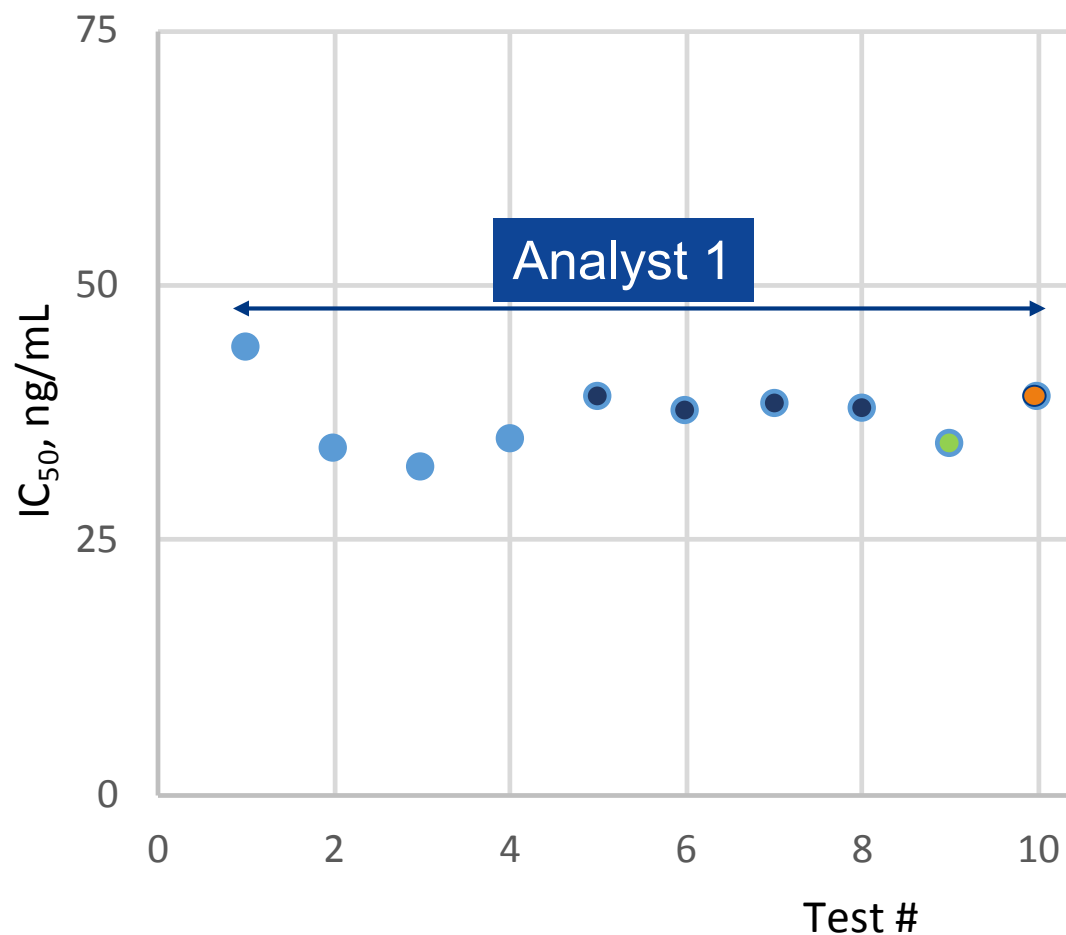
Cryopreserved (RTU)



Comparable Performance to Continuous Culture Format

Format	HillSlope	IC ₅₀ (ng/mL)	S/B
Continuous Culture	-2.337	59.1	28
Cryopreserved (RTU)	-2.264	36.8	20

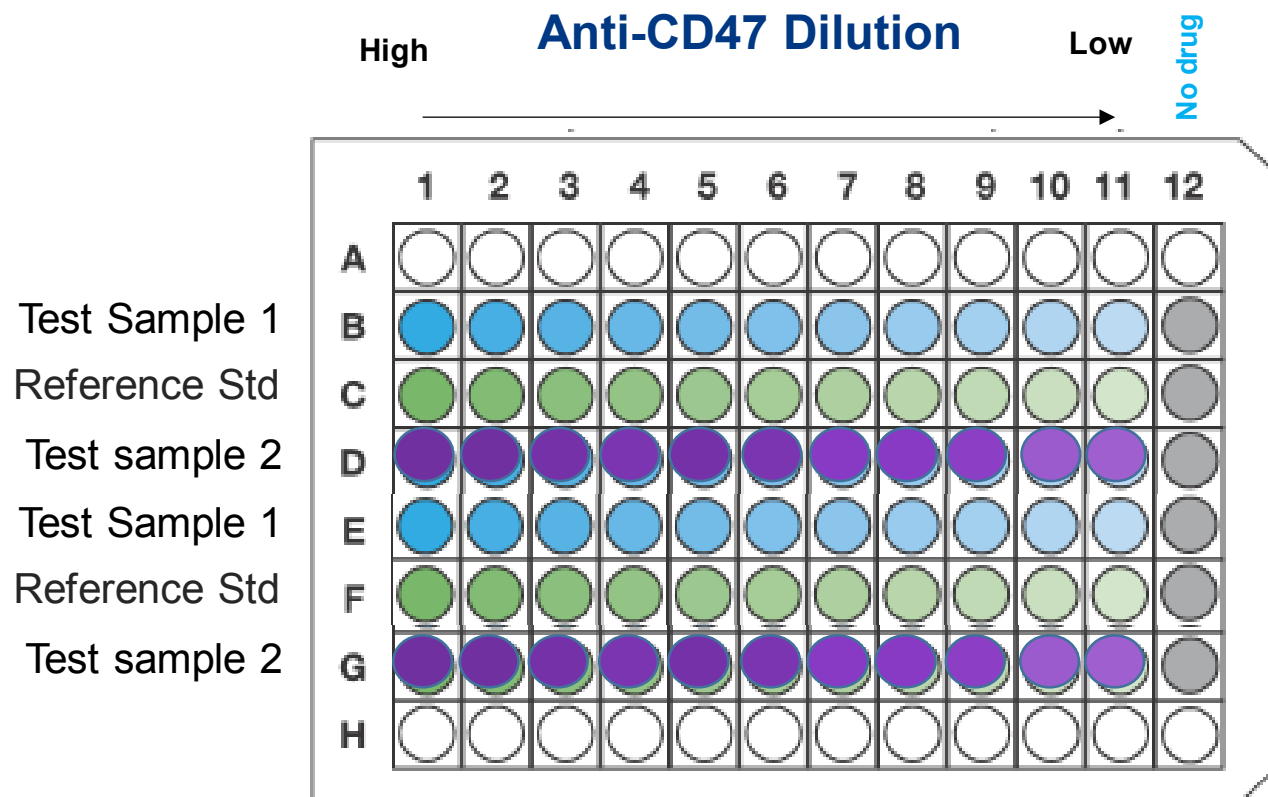
Multiple SIRP α Bioassay Lots Demonstrate Low Inter-Lot and Inter-Run Variability for Co-Culture Assay



- SIRP α Lot A / CD47 Lot A
- SIRP α Lot B / CD47 Lot B
- SIRP α Lot A / CD47 Lot B
- SIRP α Lot B / CD47 Lot A

- For each analyst, observed excellent inter-lot and inter-run reproducibility in IC₅₀
- More variability in IC₅₀ between analysts
- Variation between analysts not expected to impact relative potency measurements

Example plate layout

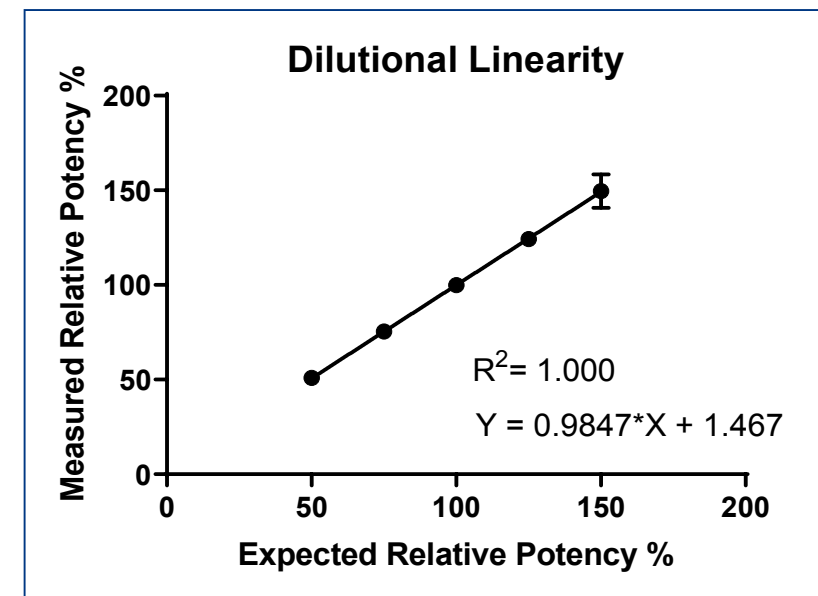


- Two analysts, multiple days
- 5 sample concentrations over range of 50-150% (50%, 75%, 100%, 125% and 150%)
- Each concentration evaluated 3 times by each analyst over a minimum of 3 days
 - Each sample tested in duplicate wells per dose with interleaved plate layout
- Specificity and forced degradation samples included

SIRPα Bioassay Qualification:

Excellent Accuracy and Dilutional Linearity Over Range of 50-150%

Expected RP (%)	Exp #	Analyst #	Measured RP (%)	Average RP (%)	% RSD	% Accuracy	Relative Bias, %
150	1	1	164	149.5	5.96	99.7	-0.3
	2	1	144				
	3	1	145				
	4	2	140				
	5	2	148				
	6	2	156				
125	1	1	123	124.2	3.16	99.4	-0.6
	2	1	125				
	3	1	124				
	4	2	119				
	5	2	123				
	6	2	131				
100	1	1	102	99.8	3.66	99.8	0.2
	2	1	95				
	3	1	103				
	4	2	104				
	5	2	98				
	6	2	97				
75	1	1	75	75.3	5.15	100.4	0.4
	2	1	73				
	3	1	79				
	4	2	73				
	5	2	81				
	6	2	71				
50	1	1	55	50.8	6.51	101.6	1.6
	2	1	52				
	3	1	53				
	4	2	51				
	5	2	48				
	6	2	46				



Accuracy	100.02%
Intermediate precision	6.5%
Relative Bias	1.6%
Dilutional Linearity	$R^2 = 1.000$
Range	50-150%

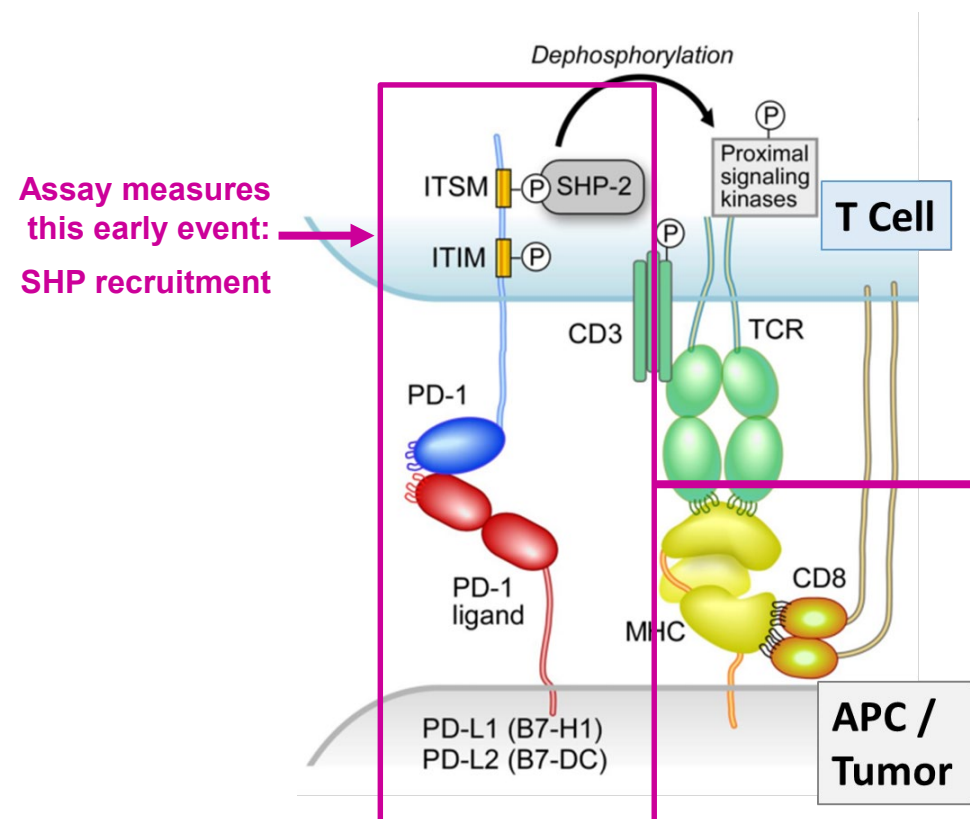
Case Study 2

Feasibility and Qualification of PathHunter® PD-1 Signaling Assay at Two Labs: Eurofins DiscoverX and Sartorius Stedim

PD-1 Signaling Assay Concept

Quantify early step in PD-1 mediated inhibition of T cell activation: SHP recruitment

Mechanism of Action



Assay Design

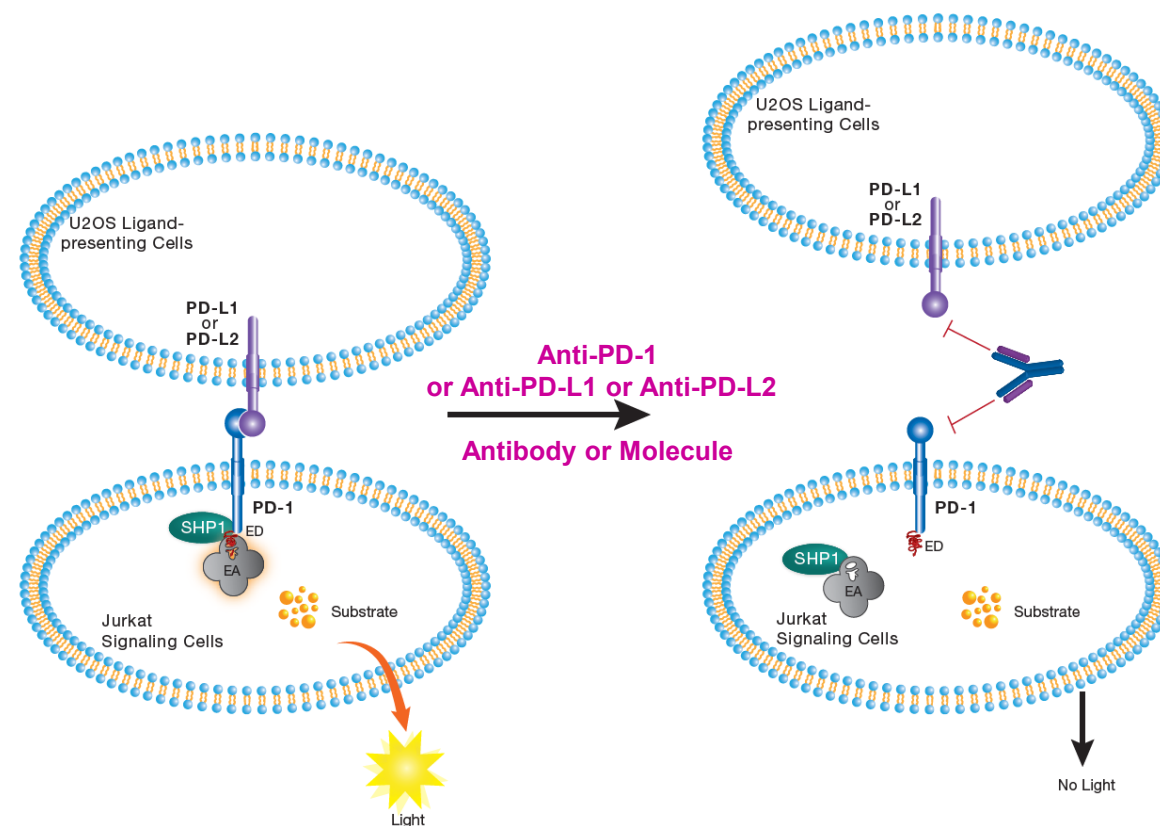
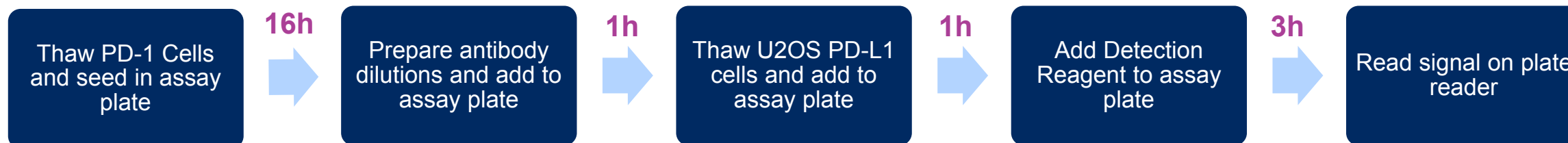


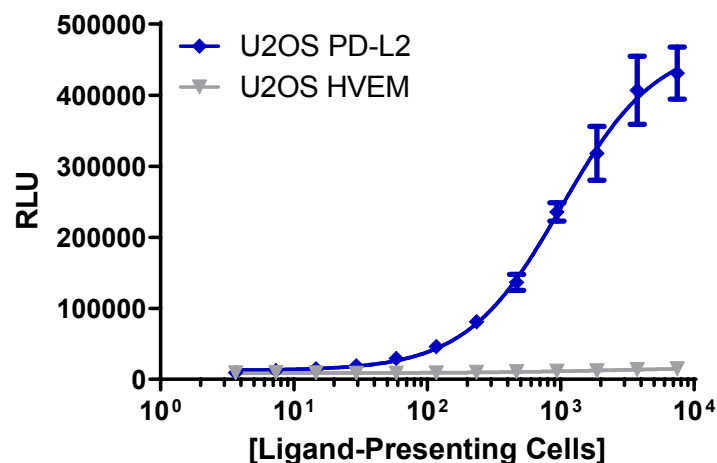
Figure from Science Webinar Series, Part 5: Gordon J. Freeman, Ph.D.

PD-1 Signaling Assay is Highly Specific and Stability-Indicating

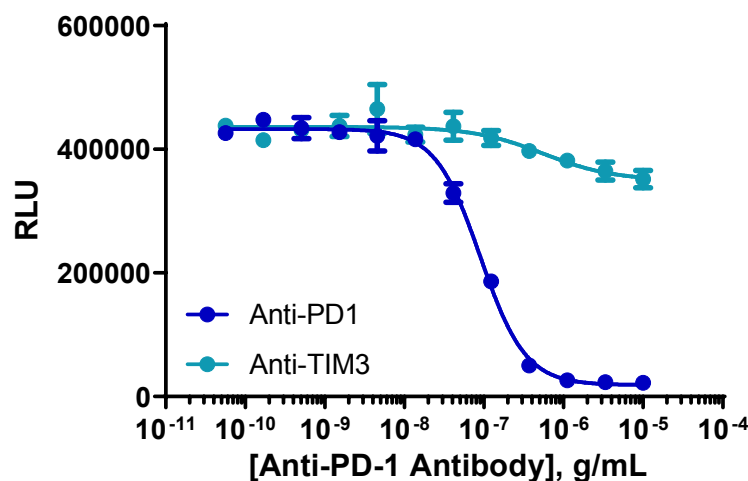
RTU (Bioassay) Method



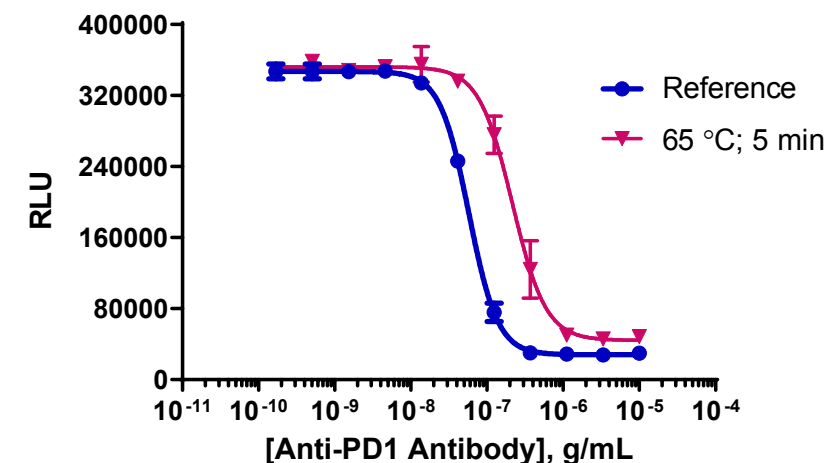
Specificity (Agonist)



Specificity (Antagonist)



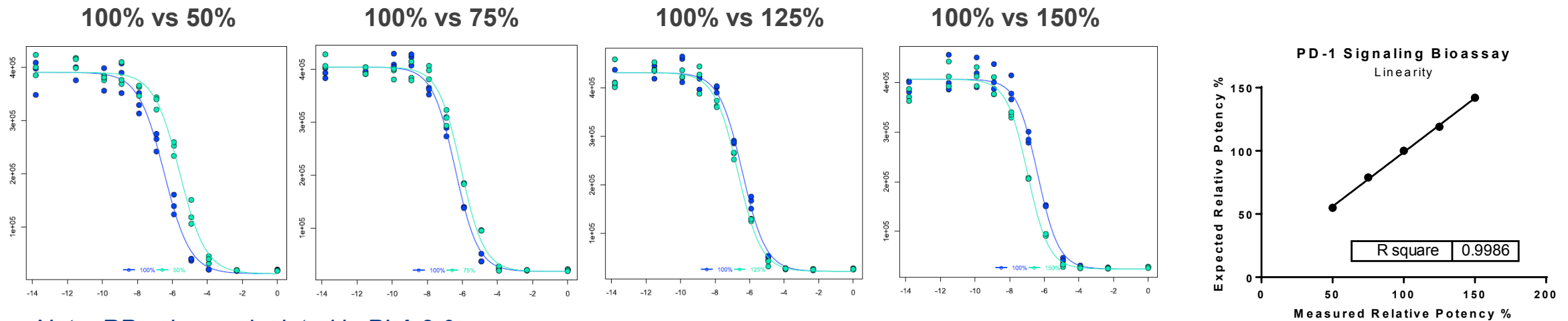
Stability-Indicating



Feasibility Study with Keytruda® and Opdivo® in the PathHunter® PD-1 Signaling Assay

Initial qualification performed with 5 drug concentrations (50%, 75%, 100%, 125%, 150%) by a single analyst over multiple days with both Pembrolizumab and Nivolumab, with a minimum of 3 tests per concentration

Example Relative Potency Data for Pembrolizumab



Note: RP values calculated in PLA 3.0

Drug	Accuracy	Precision	Linearity
Keytruda (Pembrolizumab)	99.7%	4.8%	0.994
Opdivo (Nivolumab)	100.5%	6.0%	0.998

Keytruda is a registered trademark of Merck Sharp & Dohme Corp., a subsidiary of **Merck & Co., Inc**
Opdivo is a registered trademark of Bristol Myers Squibb

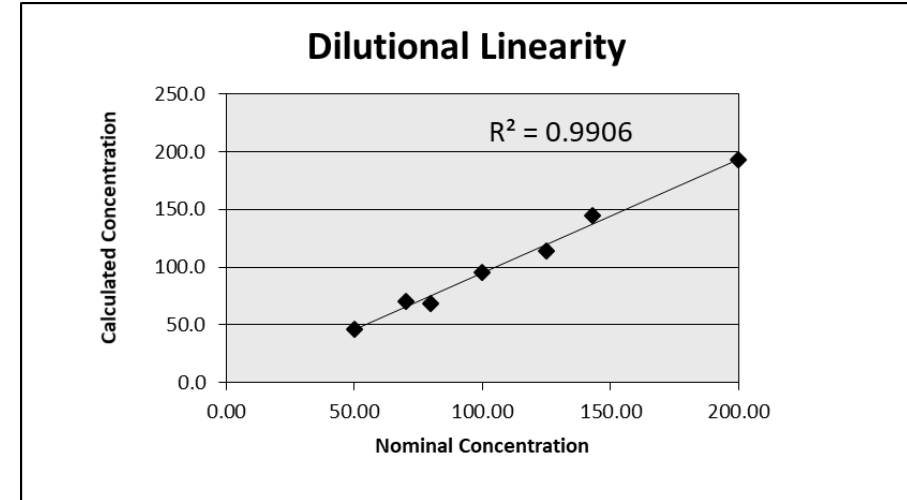
PD-1 Bioassay Qualification Study Design for Nivolumab (Sartorius Stedim)

Study Design per ICH Guidelines Covering Range of 50-200%

Assessment #	Operator	Kit Lot#	Samples (%)	
1	A	1	50	100
2	B	1	50	200
3	B	1	100	Specificity
4	C	1	200	50
5	A	1	100	200
6	B	2	143	70
7	D	2	Heat-Treated 100%	Freeze/Thaw 100%
8	C	2	200	Heat-Treated 143%
9	E	2	80	125

PD-1 Bioassay Qualification Results (Sartorius Stedim)

Nominal Concentration	Calculated Concentration	% Accuracy	Inaccuracy
50	46.1	92.2%	-7.8
50	45.7	91.4%	-8.6
50	47.5	95.0	-5.0
70	69.9	99.9	-0.1
80	68.4	85.5	-14.6
100	88.0	88.0	-12.0
100	97.3	97.3	-2.7
100	101.1	101.1	1.1
125	114.3	91.4	-8.6
143	144.3	100.9	0.9
200	197.1	98.6	-1.5
200	190.6	95.3	-4.7
200	190.4	95.2	-4.8
Specificity	0.7	N/A	N/A



Accuracy: within 14.6% across a range of 50- 200%

Intermediate precision: within 7% CV

Dilutional linearity: $R^2 = 0.9906$

Low variability and good reproducibility between different analysts and over multiple days

- Qualified MOA-reflective bioassays have been established for two important immunotherapy targets, PD-1 and SIRP α (CD47)
- Both assays were accurate and precise:
 - For PD-1: accuracy within 14.5% across a range of 50 to 200% and intermediate precision within 7% CV
 - For SIRP α : accuracy within 1.6% across a range of 50-150% and intermediate precision within 6.5% RSD
- Both assays demonstrated excellent dilutional linearity: R^2 value of 0.9906 for PD-1 and 1.000 for SIRP α
- Low variability and good reproducibility were observed between analysts and on different days
- Assays are suitable for QC lot release due to low variability, good accuracy and precision
- Ready-to-use format and optimized protocol transferred easily between sites for rapid implementation
- Assay-ready formats of Eurofins DiscoverX assays are available as off-the-shelf products or can be customized for your candidate molecule

Thank You for Tuning In!

Acknowledgements:

Sartorius Stedim BioOutsource Ltd

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Lisa Blackwood

Eurofins DiscoverX

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Hyna Dotimas	LiCi Zhu
Ai Shih	Neil Charter
Jennifer Lin-Jones	

For more information about our Products:
discoverx.com/bioassay

For questions or to speak to an expert, contact:
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