



# A Novel MOA-reflective Bioassay for Quantifying Potency of Therapeutics Targeting the SIRP $\alpha$ |CD47 Signaling Axis

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*Director R&D, Eurofins DiscoverX*

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CD47 Summit 2020 (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 & assay development**
- **Bioassay development, optimization & qualification**
- Analytical Cell Banks
- Membrane Preps and Frozen Assay Ready Cells
- Bulk Enzyme Production

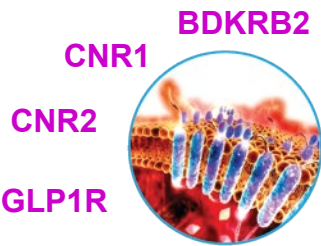
### Established Brand

Successfully implemented at global Pharma, Biotech & CRO

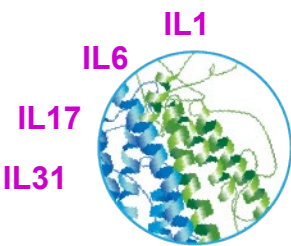
- **Products implemented in discovery & development**
- Over 50 billion data points screened
- **2,000+ publications**
- Several active Biotech/CRO-partnered programs
- **Implemented in lot release of several marketed biologics in US and EU**

# Cell-Based Assays Supporting Discovery to Lot Release Screening, Characterization, Potency, & NAb Assay Development

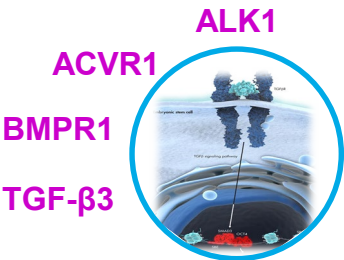
Industry’s largest menu with 800+ cell lines to support bioassay development for major drug target classes



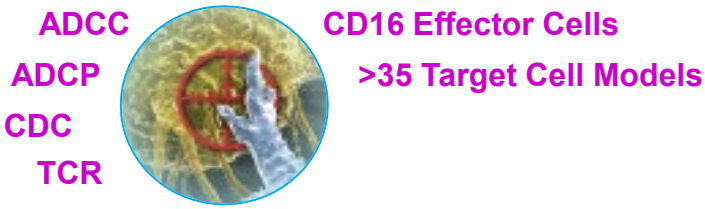
GPCRs



Interleukins

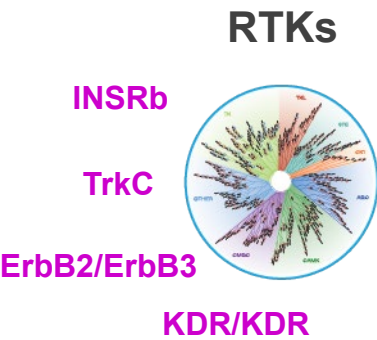


TGFβ Superfamily

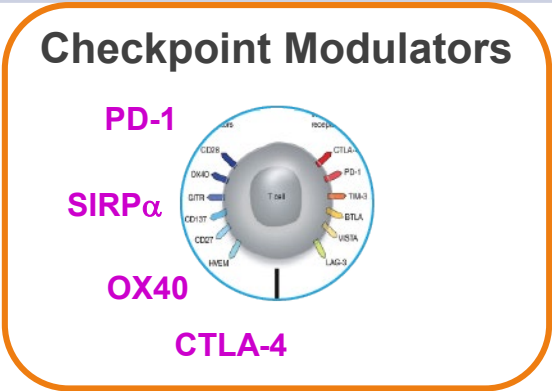


Cytotoxicity

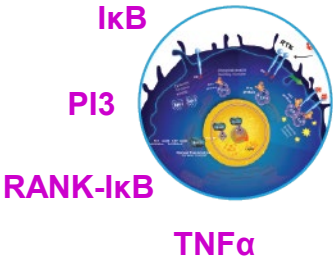
Available Targets	289	55	50	38	16	30+	43	58 (22)
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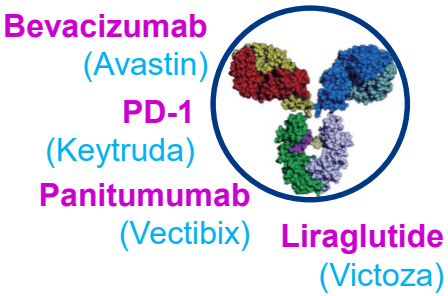
RTKs



Checkpoint Modulators



Pathway

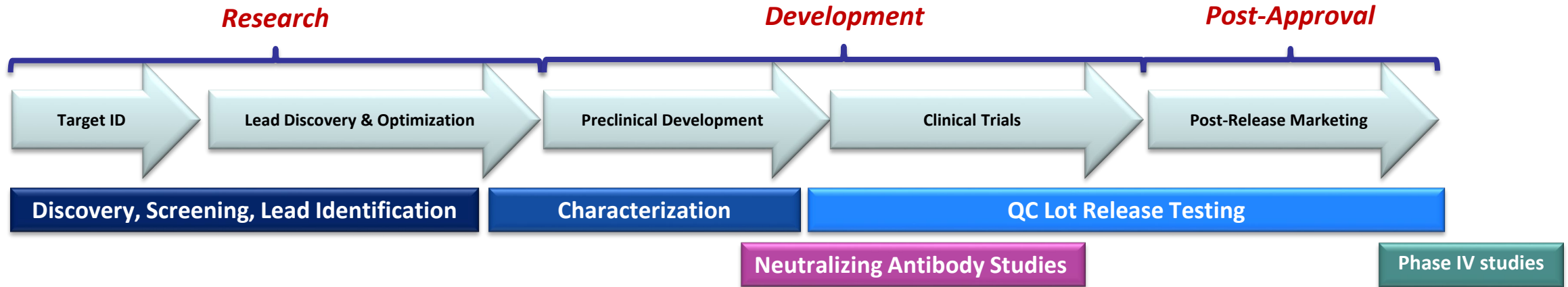


Bioassays

Qualified with innovator drug

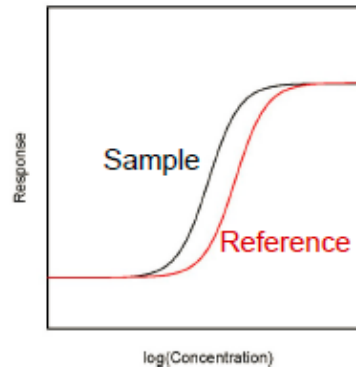
# Requirements for Cell-Based Assays in Late-Stage Development of Biologics

*Cell-based assays must be fit-for-purpose*



## Assays for Lot Release , Stability Testing

- Use**
- Relative potency, characterization, process development
- 
- Requirements**
- MOA-reflective
  - Robustness
  - Accuracy
  - Intermediate Precision
  - Linear (over a range of 50-150%)
  - Stability-indicating
  - Amenable to transfer to multiple sites

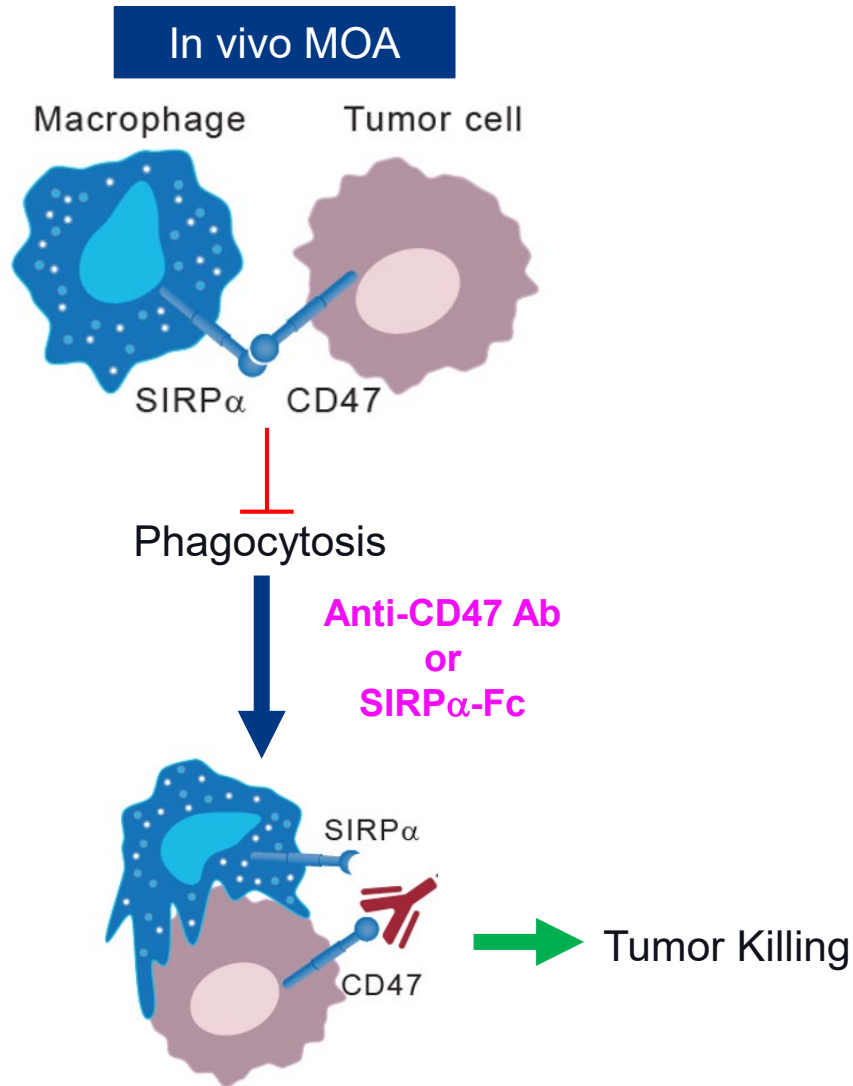


- Implementation**
- Emphasis on engineered cell models rather than primary cells
  - Assay-ready cells rather than continuous culture
  - Robust and easy-to-transfer protocols



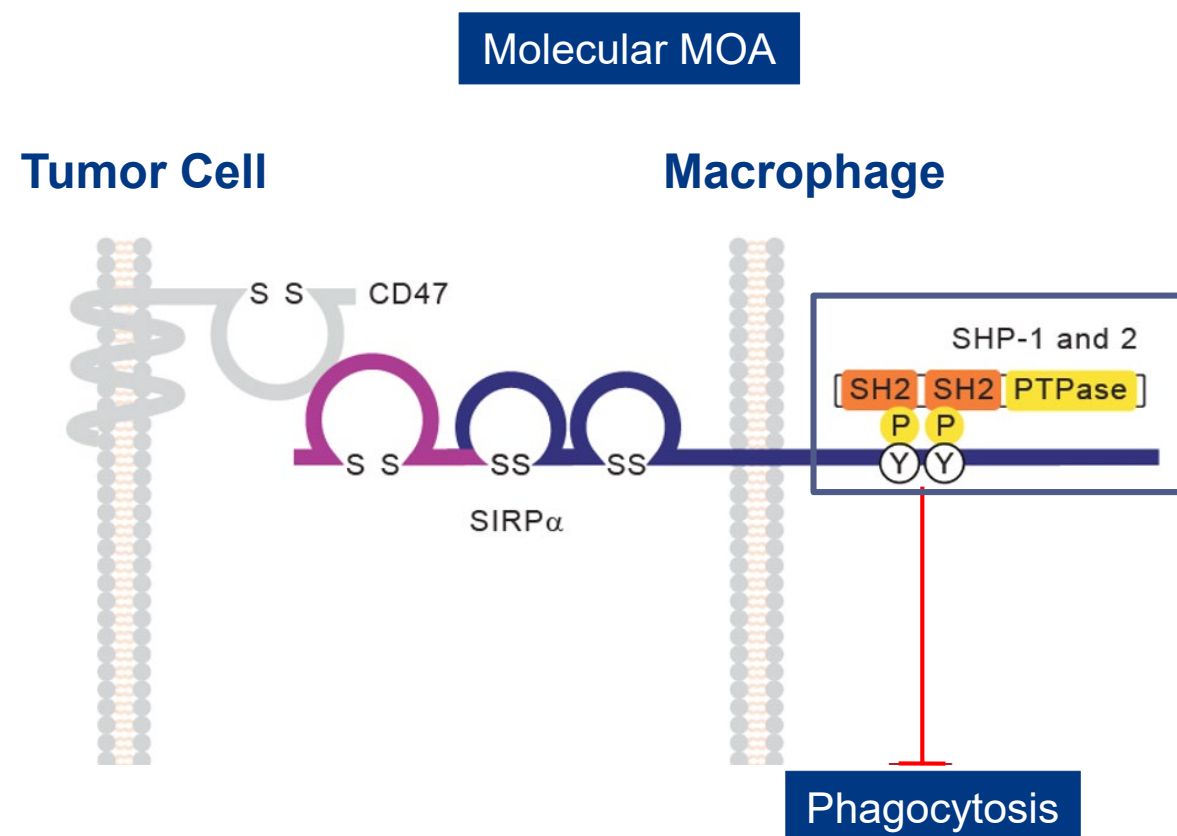
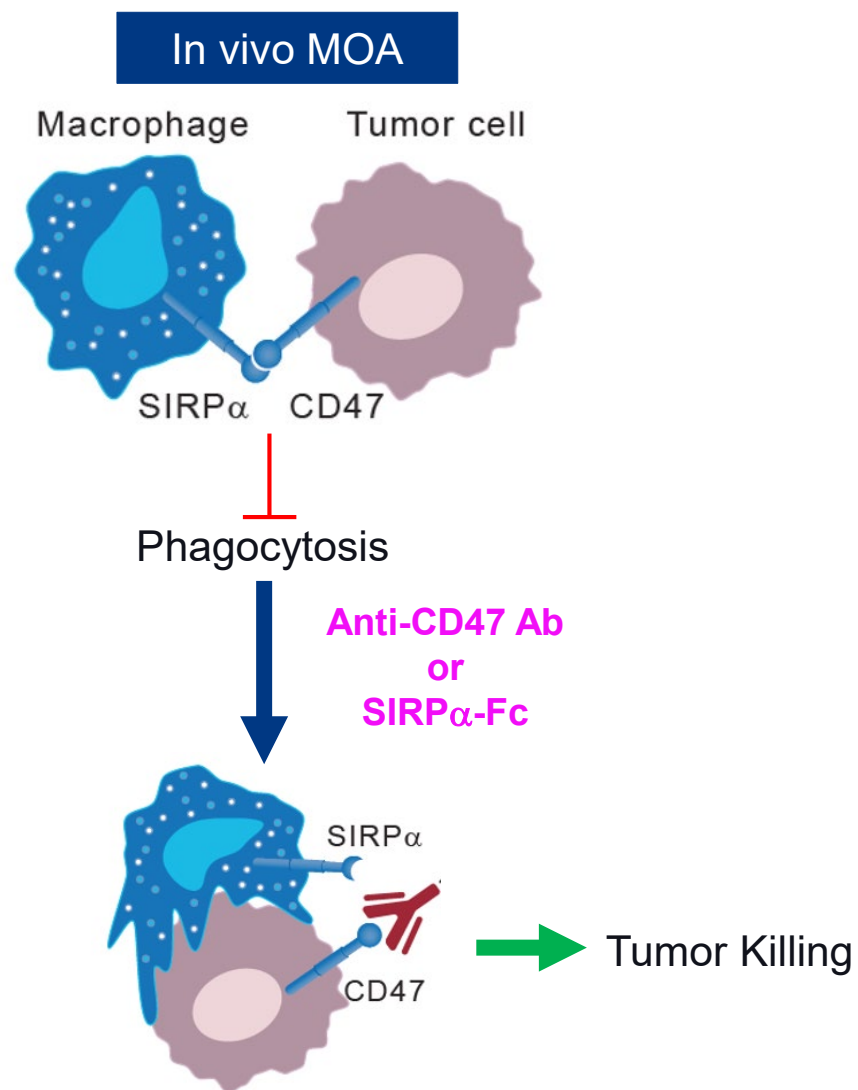
# The SIRP $\alpha$ / CD47 Axis

## An Innate Immune Checkpoint



- SIRP $\alpha$  is an inhibitory receptor expressed on macrophages and dendritic cells that promotes phagocytosis of foreign objects
- CD47, the ligand for SIRP $\alpha$ , 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 $\alpha$ , preventing myosin-IIA accumulation at the phagocytic synapse, leading to inhibition of phagocytosis
- Blocking the CD47 / SIRP $\alpha$  axis (e.g. with anti-CD47 antibodies, engineered receptor decoys, anti-SIRP $\alpha$  antibodies and bispecific agents) promotes tumor killing
  - 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)

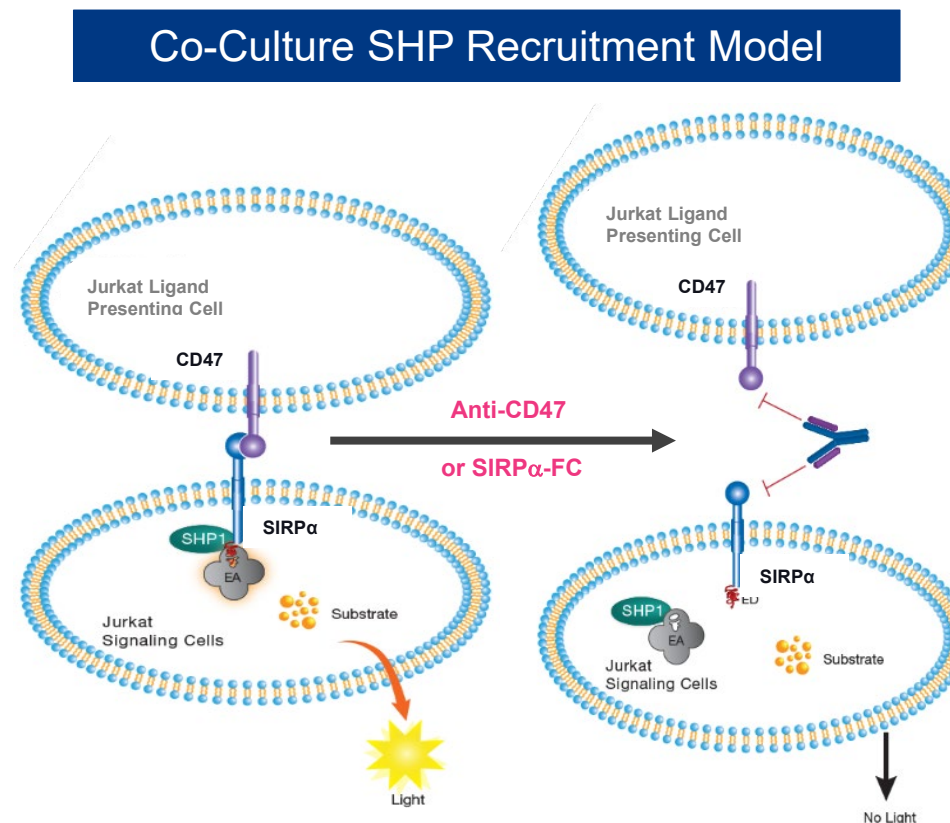
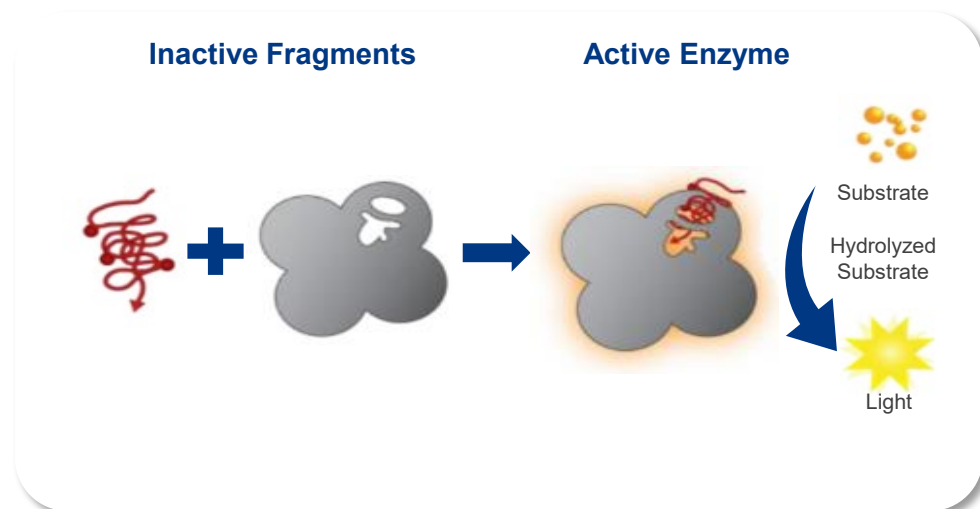
# Molecular MOA of SIRP $\alpha$ / CD47 Signaling Axis



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

# PathHunter® SIRP $\alpha$ Signaling Assay: Assay Concept

*Co-culture SHP recruitment model based on  $\beta$ -galactosidase enzyme fragment complementation*



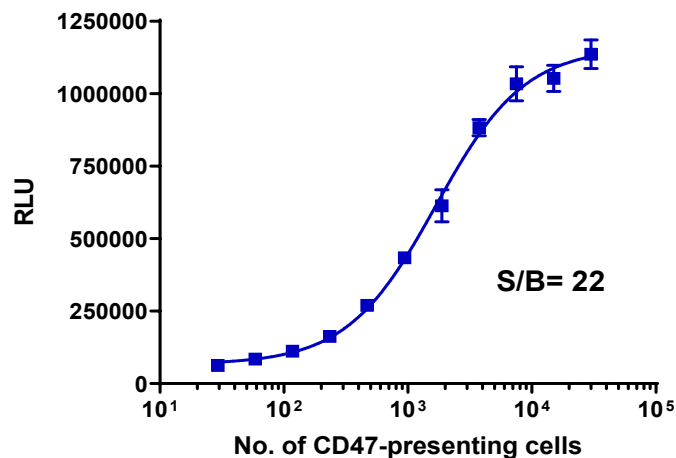
Assay quantifies ligand-induced recruitment of SHP-1 to ITIM motifs in C-terminal tail of SIRP $\alpha$  in response to phosphorylation

# PathHunter® SIRPα (CD47) Signaling Assay

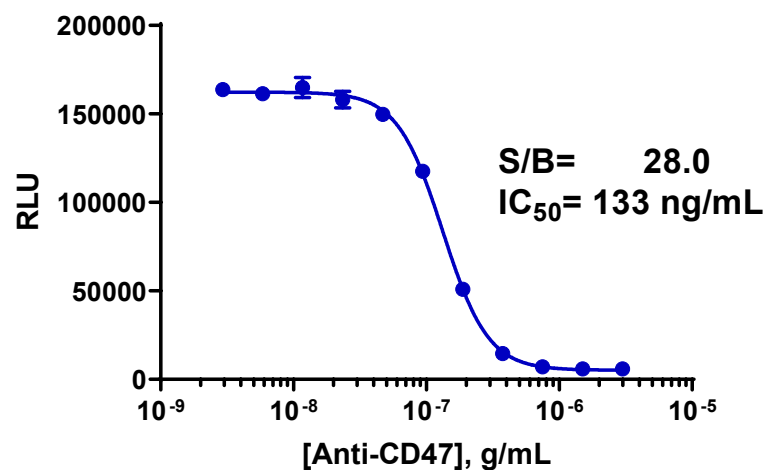
*Co-culture model with stable surface expression of SIRPα and a stable functional response over 45+ passages*

## Cell-Presented Ligand

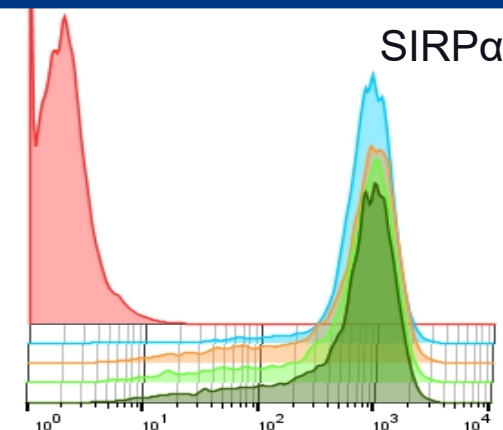
Agonist



Antagonist

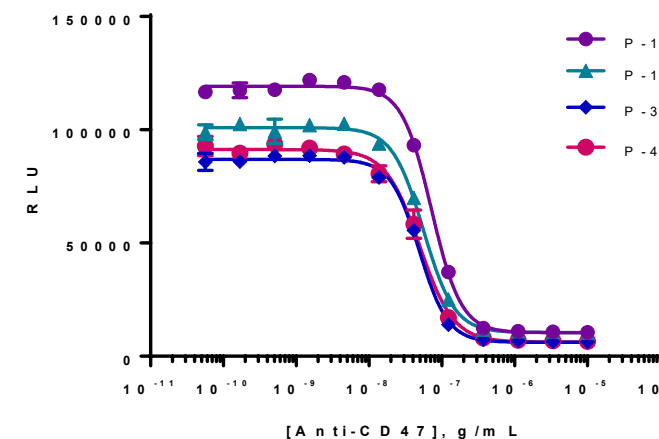


## Stable SIRPα Surface Expression



SIRPα expression varies by <20% RSD over 45 passages

## Stable Functional Response



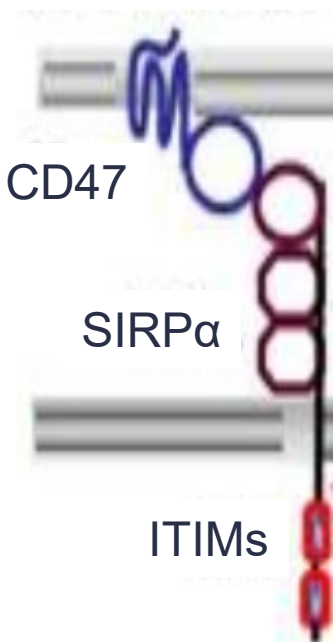
S/B : 17% RSD over 45 passages

$IC_{50}$  : <19% RSD over 45 passages



# Mutation of SIRP $\alpha$ ITIM Motifs Disrupts CD47-Mediated Signaling

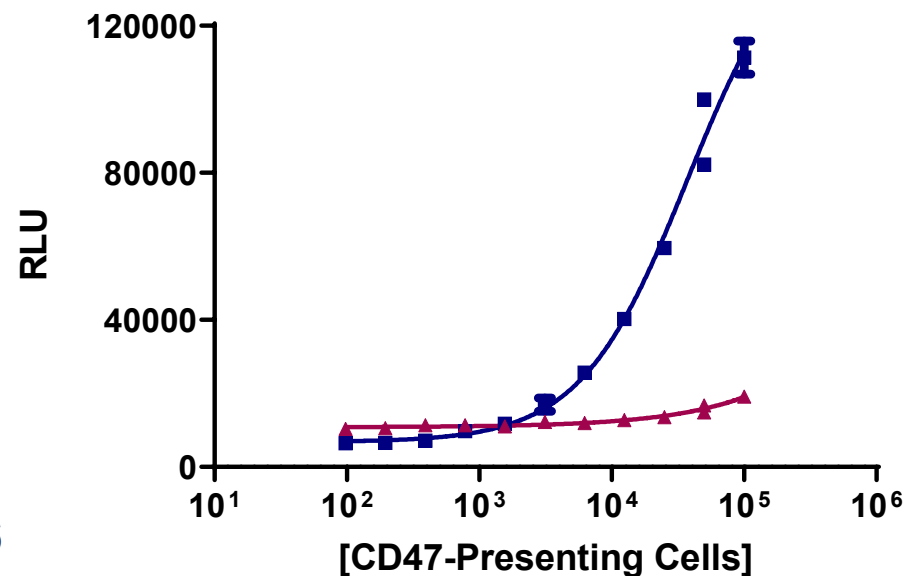
Evaluation of impact of mutations in the 4 tyrosine residues that are potential sites for phosphorylation: Y429, Y459, Y470 and Y496



Adapted from Zen, K. et al. Nat Commun 2013; 4: 2436

Agonist

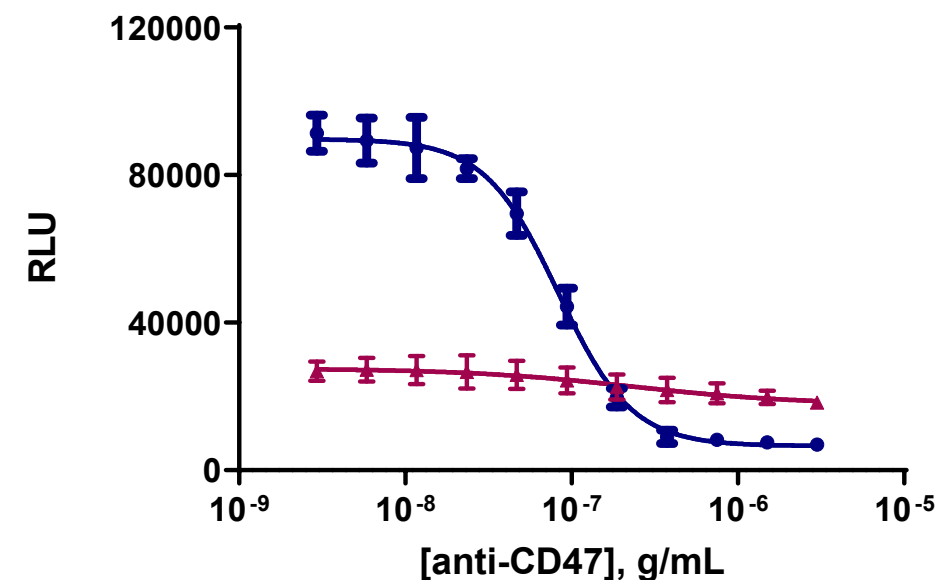
Jurkat SIRP $\alpha$  Signaling Assay



— SIRP $\alpha$  (Y429F, Y459F, Y470F, Y496F)  
— W/T SIRP $\alpha$

Antagonist

Jurkat SIRP $\alpha$  Signaling Assay

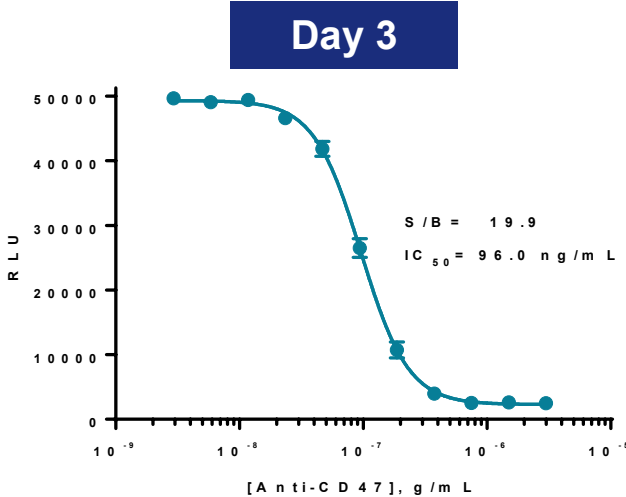
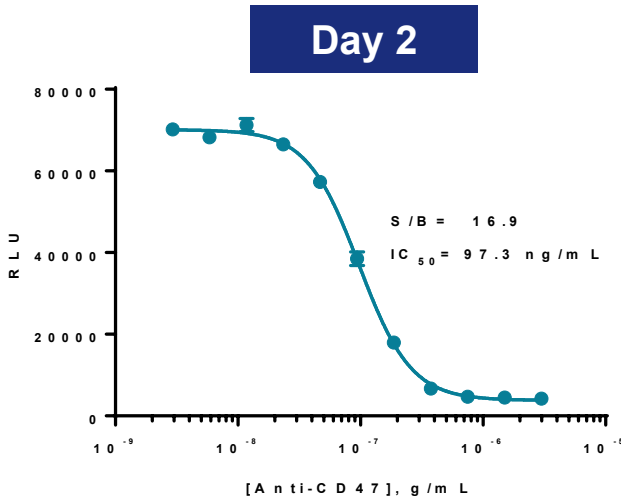
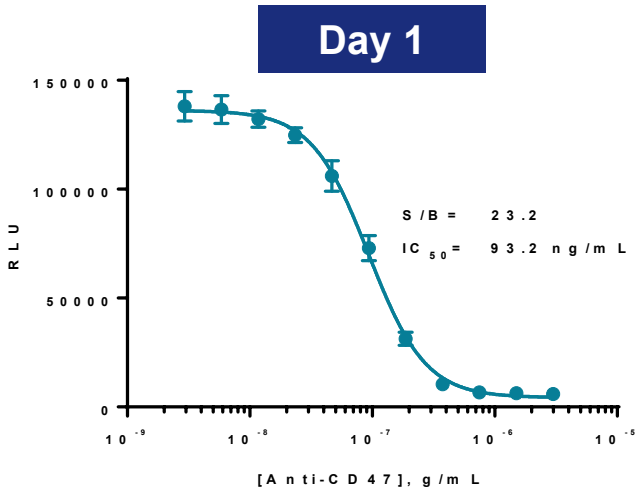


— SIRP $\alpha$  (Y429F, Y459F, Y470F, Y496F)  
— W/T SIRP $\alpha$

*Single mutations disrupted signaling to different degrees (data not shown), but mutation of 3 or more tyrosine residues completely abrogated CD47-mediated SHP recruitment*

# PathHunter SIRPα Signaling Assay: Excellent Assay Reproducibility and Intermediate Precision

Single  
Analyst  
(Multi-Day)



Analyst	Day	Estimated RP, %, Plate 1	Estimated RP, %, Plate 2	Relative Bias, %
1	1	97.8	103.2	0.5
1	2	103.6	105.7	4.65
1	3	96.5	96.8	-3.35
2	1	97.8	97.3	-2.45
2	2	98.1	98.2	-1.85

Relative  
Potency

Average Relative Potency (RP): 99.5%  
Relative Bias: 0.55%  
Intermediate Precision: 3.34%

# Development of RTU Assay Format for SIRP $\alpha$ Signaling Assay, with an Easy-to-Transfer Method

## RTU (Bioassay) Method

Thaw and add CD47 and SIRP $\alpha$  frozen cells (1 vial each) to assay plate



Prepare antibody dilutions and add to assay plate

24h



Add Detection Reagent to assay plate

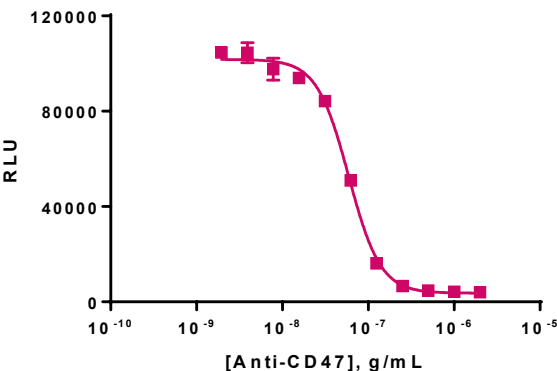
1h



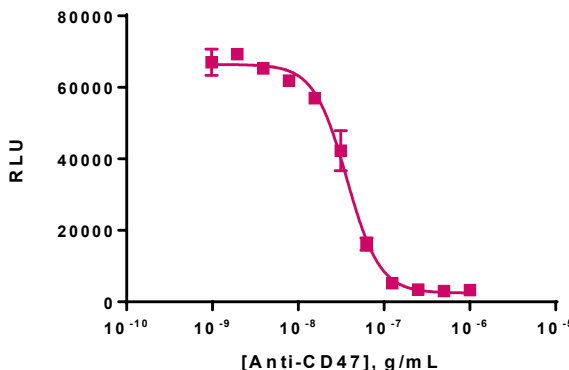
Read signal on plate reader

**Total Assay Time:**  
~26 hours

## Continuous Culture



## Cryopreserved (RTU)



## Comparable Performance to Continuous Culture Format

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

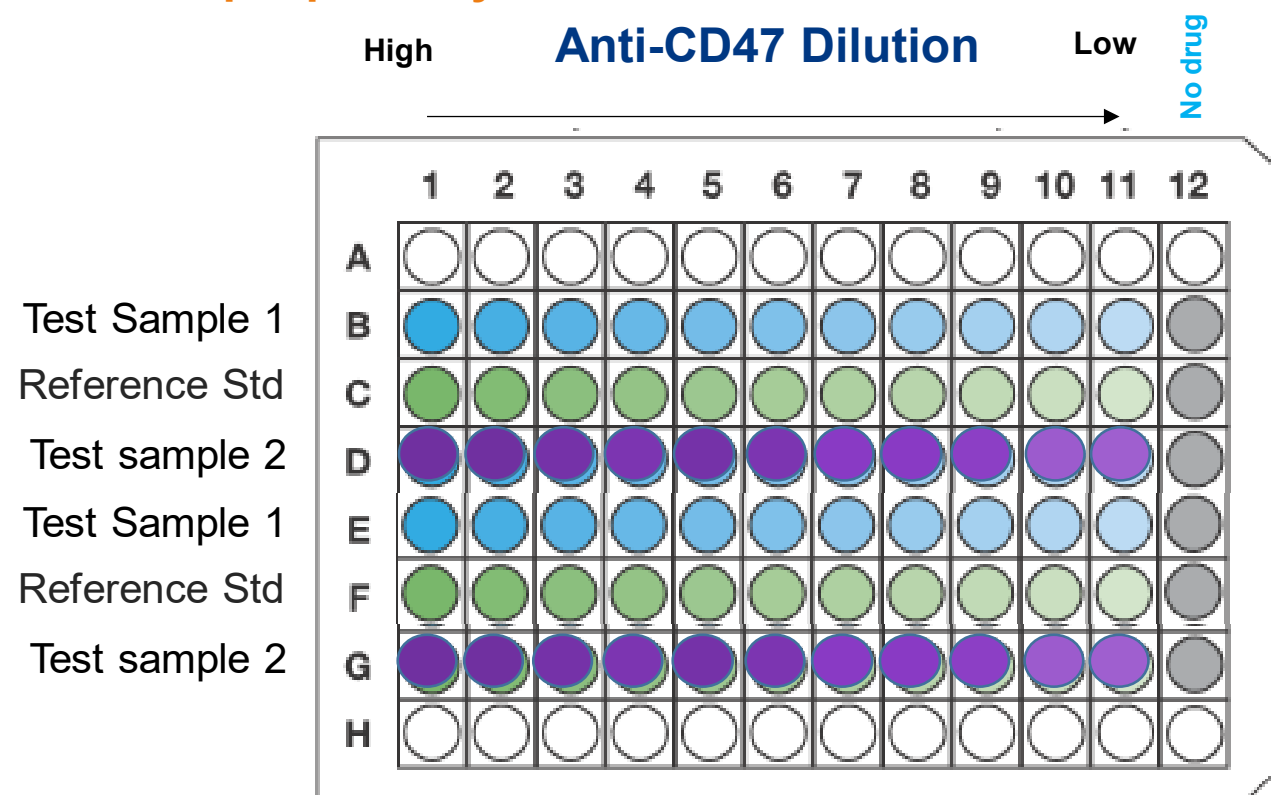
# SIRPα Bioassay Qualification: Study Design

*To establish suitability of the assay for use in lot release, we performed a qualification study based on ICH guidelines to establish the accuracy, precision, linearity and stability-indicating properties of the PH Jurkat SIRPα bioassay*

## Study Design

- Two analysts, multiple days
- 5 sample concentrations over a 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

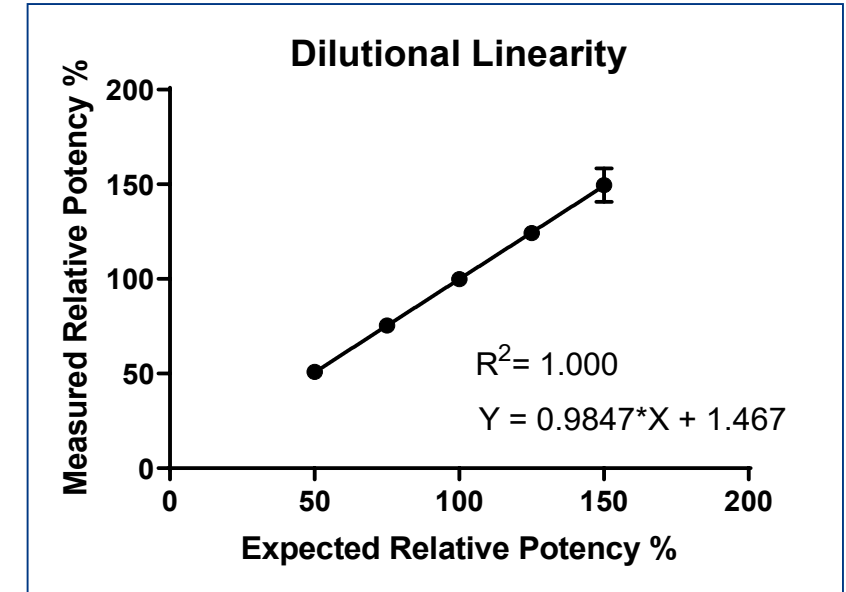
## Example plate layout



# 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				

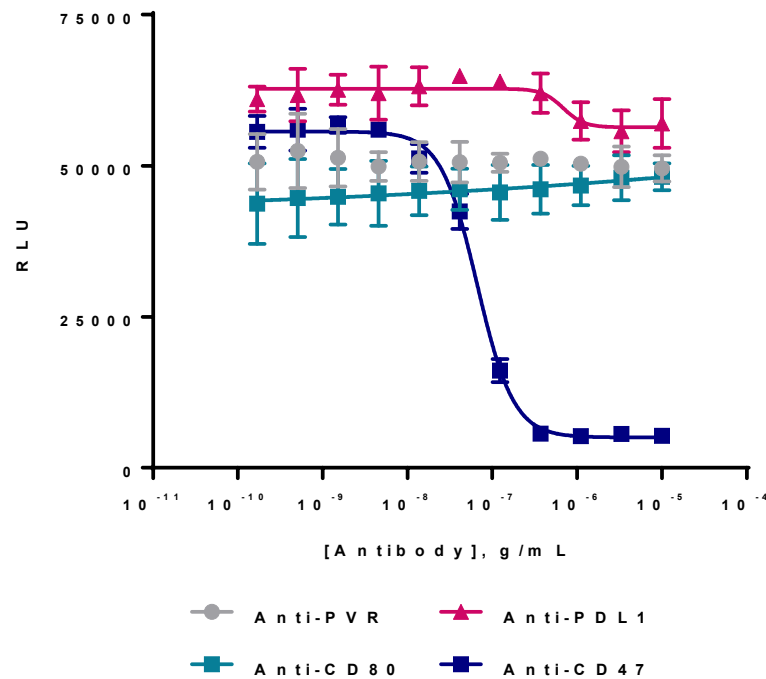


<b>Accuracy</b>	<b>100.02%</b>
Intermediate precision	6.5%
Relative Bias	1.6%
Dilutional Linearity	$R^2 = 1.000$
Range	50-150%

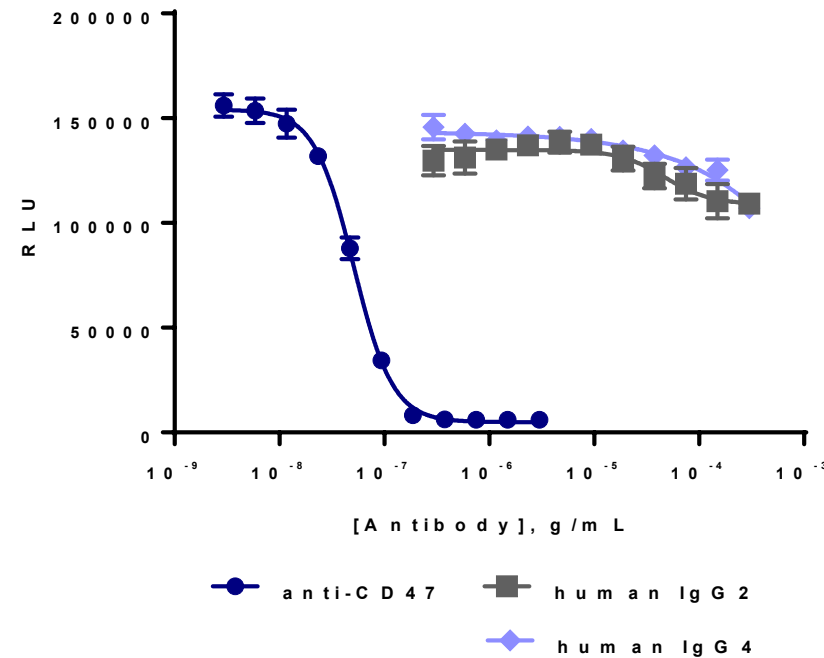


# PathHunter SIRP $\alpha$ Signaling Assay: Excellent Specificity and Stability-Indicating Properties

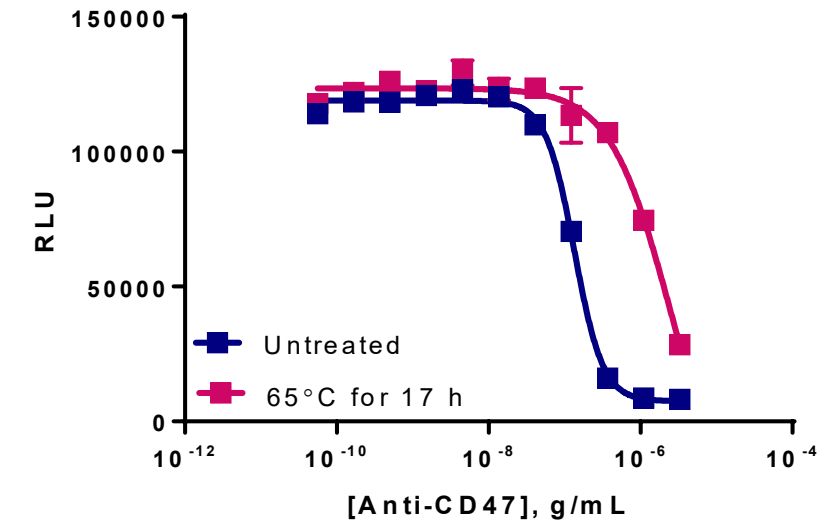
Assay Specificity



Assay Specificity

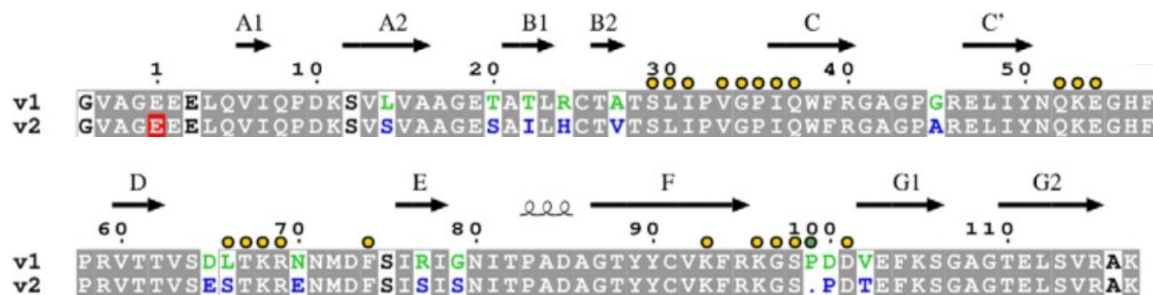


Stability-Indicating



# Signaling Assays for Most Common SIRP $\alpha$ Variants: V1 and V2

- At least 10 SIRP $\alpha$  variants identified
- SIRP $\alpha$  variants 1 and 2 (V1, V2) are most prevalent; differ by 15 amino acids



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J Biol Chem. 2014 Apr 4; 289(14): 10024–10028.

PMCID: PMC3974974

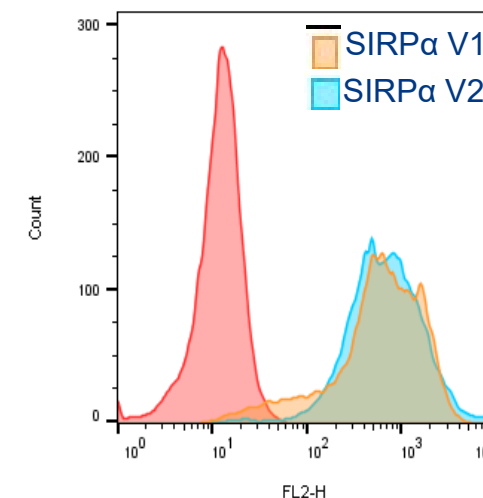
Published online 2014 Feb 18. doi: [10.1074/jbc.M114.550558](https://doi.org/10.1074/jbc.M114.550558)

PMID: [24550402](https://pubmed.ncbi.nlm.nih.gov/24550402/)

Polymorphisms in the Human Inhibitory Signal-regulatory Protein  $\alpha$  Do Not Affect Binding to Its Ligand CD47\*

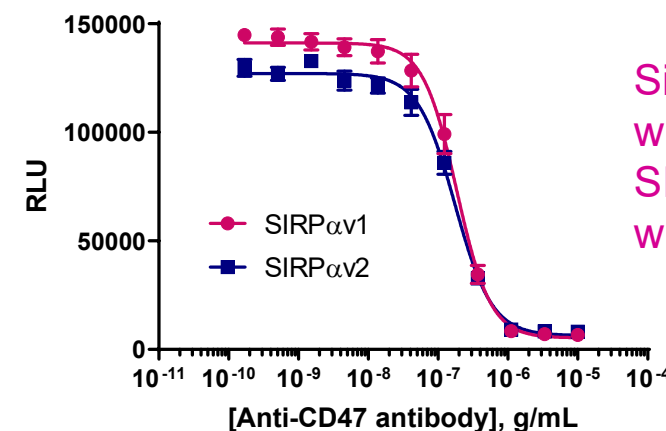
Deborah Hatherley, Susan M. Lea, Steven Johnson, and A. Neil Barclay<sup>1</sup>

## SIRP $\alpha$ Surface Expression



Comparable surface expression for SIRP $\alpha$  V1 and V2

## SIRP $\alpha$ Functional Response



Similar assay window and IC<sub>50</sub> for SIRP $\alpha$  V1 and V2 with anti-CD47

# PathHunter SIRP $\alpha$ Signaling Assay

## Ready-to-Implement solutions for your development programs

*First commercially-available SIRP $\alpha$ /CD47 cell-based assay*

We have generated an engineered cell system that measures activation and inhibition of the CD47 / SIRP $\alpha$  signaling axis without using macrophages:

- Mechanistically-relevant endpoint (SHP1 recruitment) that robustly quantifies CD47-mediated SIRP $\alpha$  signaling
  - Assays available for both SIRP $\alpha$  V1 and SIRP $\alpha$  V2 variants
  - Suitable for anti-CD47 or SIRP $\alpha$ -targeted therapeutics
- Delivers highly specific response
- Expression of SIRP $\alpha$  and functional performance are stable over >45 passages
- Excellent intermediate precision, accuracy, and dilutional linearity observed, demonstrating suitability for potency and stability applications
- Assay is tolerant to human matrix, making it suitable for neutralizing antibody detection application
- Available in continuous culture and ready-to use formats

Thank you for your attention!

## Options to suit your program needs



Purchase:  
Cell Lines / Bioassay Kits



Quick Confirmation: Test  
eXpress Kit



Proof-of-Concept:  
Feasibility Study



Cell Line Rental:  
3-months Block

Stable Cell Lines

Qualified Bioassays

MOA-based Bioassays

Analytical Cell Banks

Custom Assay Development

GPCRs

Checkpoint Receptors

Cytokine Receptors

Kinases

Signaling Pathways

TGF $\beta$  Superfamily

ADCC Assays

ADCP Assays

CDC Assays

Target Cells

Effector Cells

Technical Support: [DRX\\_SupportUS@eurofinsUS.com](mailto:DRX_SupportUS@eurofinsUS.com)