



A Novel MOA-reflective Bioassay for Quantifying Potency of Therapeutics Targeting the SIRP α |CD47 Signaling Axis

Jane Lamerdin, Ph.D.

Director R&D, Eurofins DiscoverX

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CD47 Summit 2020 (Virtual Conference)

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

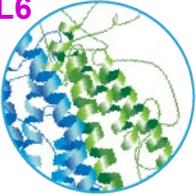
GPCRs

CNR1
CNR2
GLP1R
BDKRB2



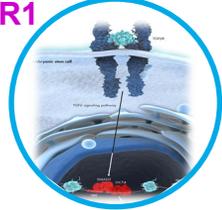
Interleukins

IL1
IL6
IL17
IL31



TGFβ Superfamily

ACVR1
BMPR1
TGF-β3
ALK1



Cytotoxicity

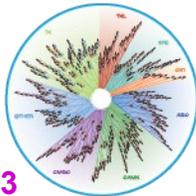
ADCC
ADCP
CDC
TCR
CD16 Effector Cells
>35 Target Cell Models



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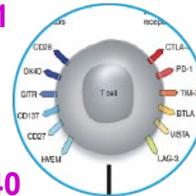
RTKs

INSRb
TrkC
ErbB2/ErbB3
KDR/KDR



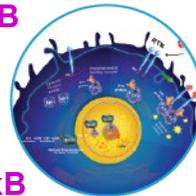
Checkpoint Modulators

PD-1
SIRPα
OX40
CTLA-4



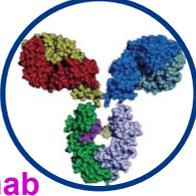
Pathway

IκB
PI3
RANK-IκB
TNFα



Bioassays

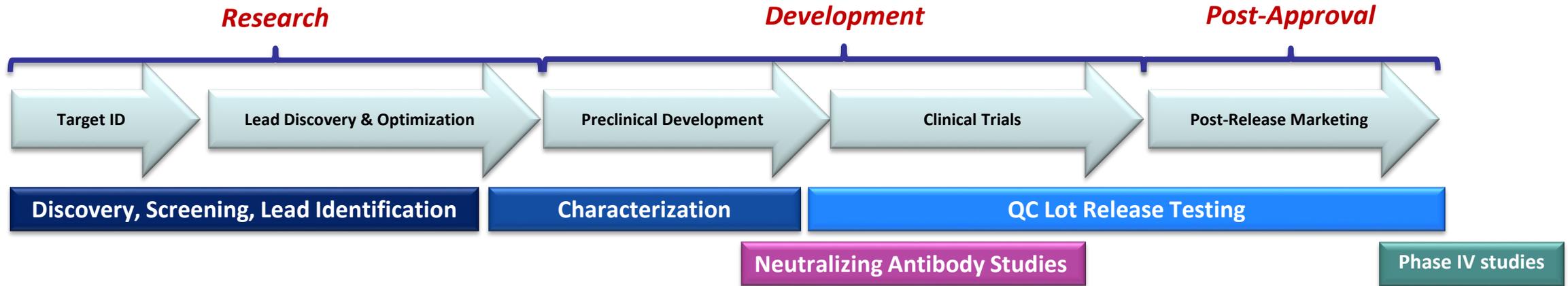
Bevacizumab (Avastin)
PD-1 (Keytruda)
Panitumumab (Vectibix)
Liraglutide (Victoza)



Qualified with innovator drug

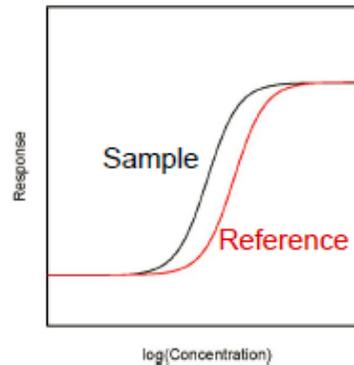
Requirements for Cell-Based Assays in Late-Stage Development of Biologics

Cell-based assays must be fit-for-purpose



Assays for Lot Release, Stability Testing

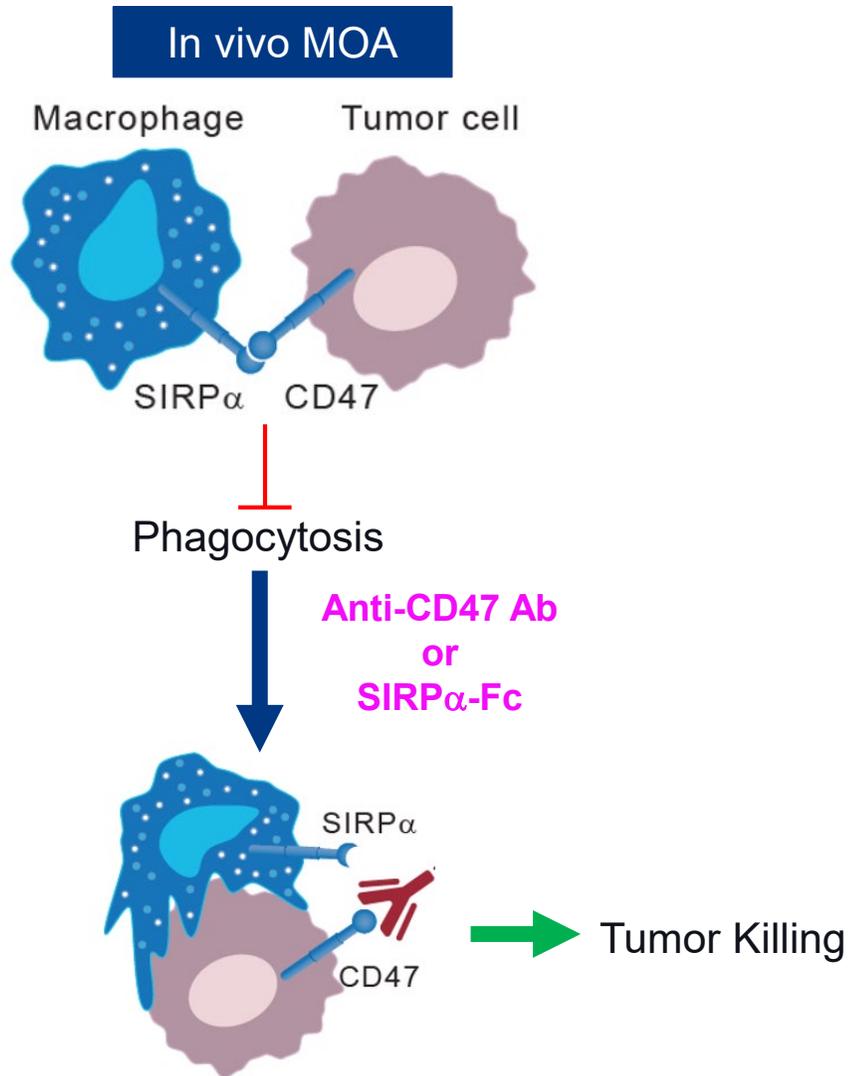
- Use**
- Relative potency, characterization, process development
-
- Requirements**
- MOA-reflective
 - Robustness
 - Accuracy
 - Intermediate Precision
 - Linear (over a range of 50-150%)
 - Stability-indicating
 - Amenable to transfer to multiple sites



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

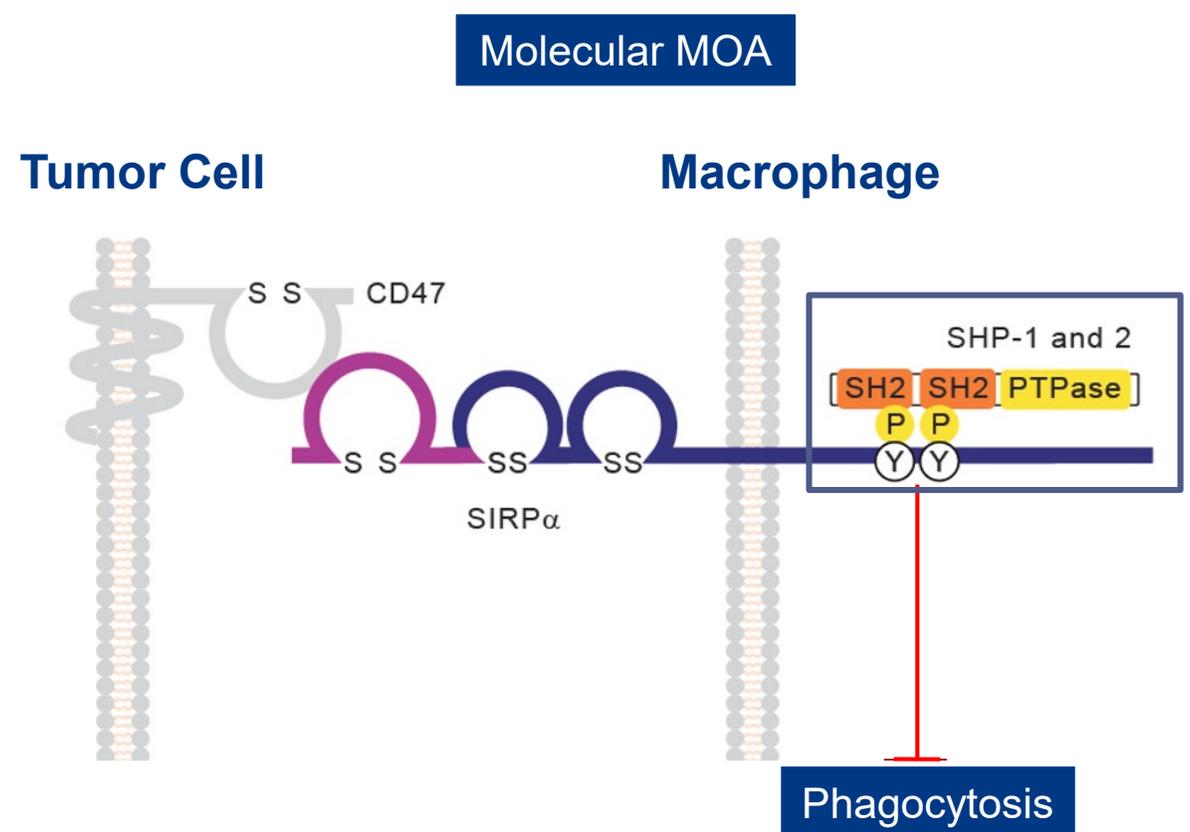
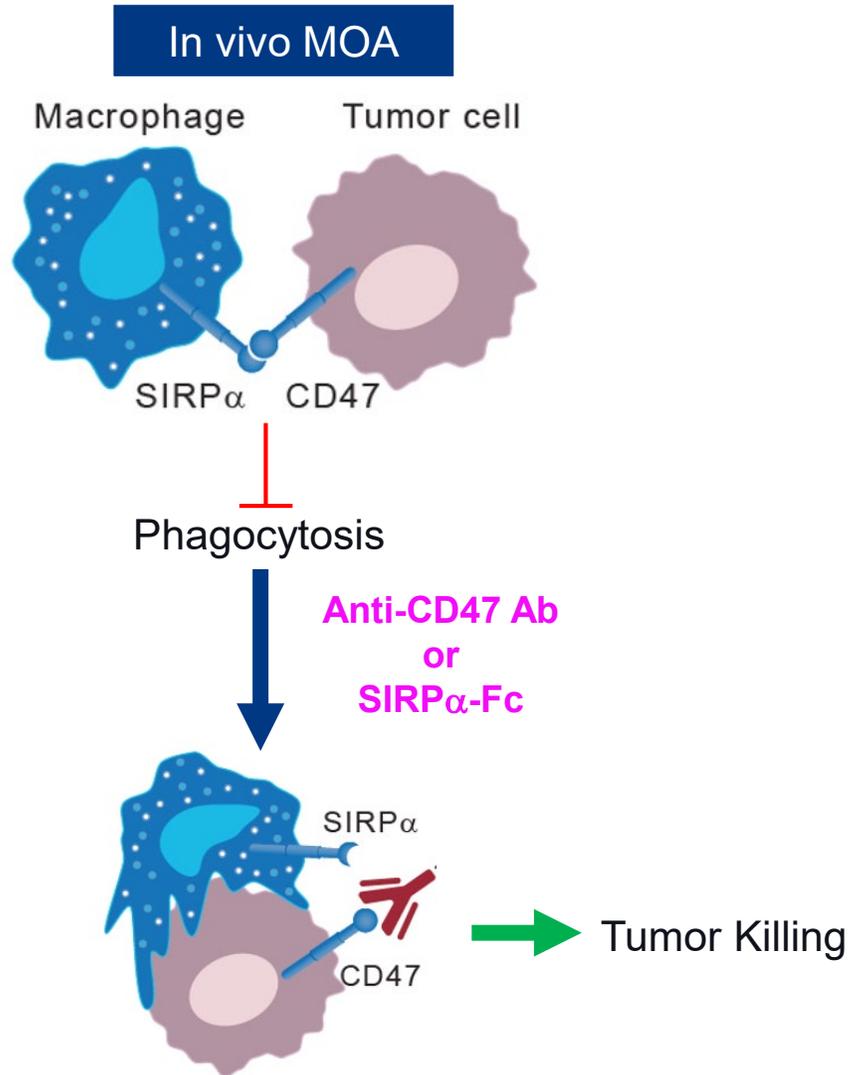
The SIRP α / CD47 Axis

An Innate Immune Checkpoint



- SIRP α is an inhibitory 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 , preventing myosin-IIA accumulation at the phagocytic synapse, leading to inhibition of phagocytosis
- Blocking the CD47 / SIRP α axis (e.g. with anti-CD47 antibodies, engineered receptor decoys, anti-SIRP α 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)

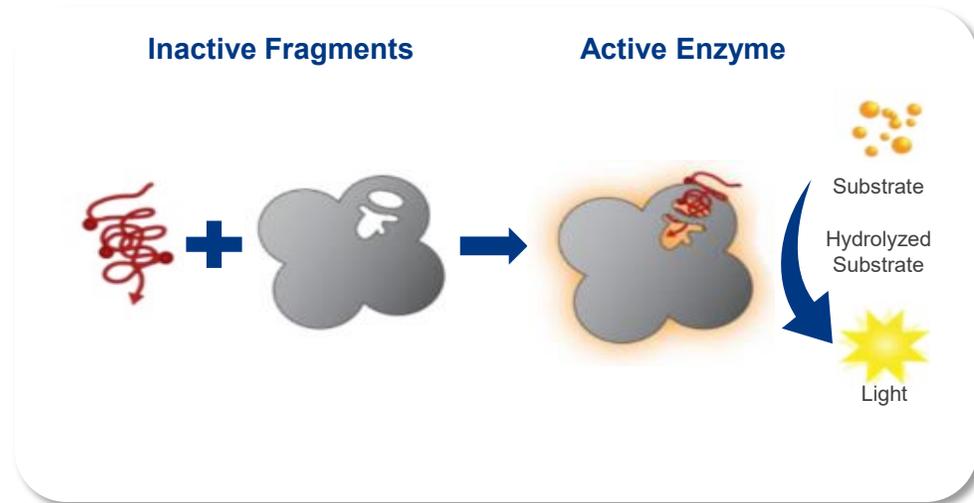
Molecular MOA of SIRP α / CD47 Signaling Axis



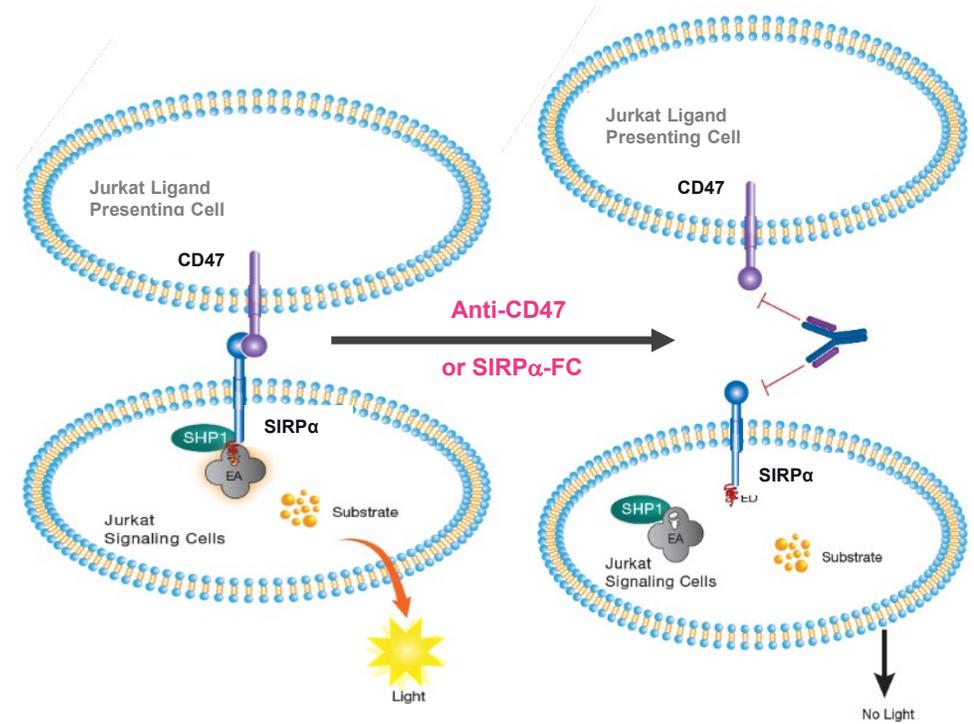
Adapted from Trends in Cell Biology, 2008. Vol 19, No. 2

PathHunter® SIRP α Signaling Assay: Assay Concept

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



Co-Culture SHP Recruitment Model



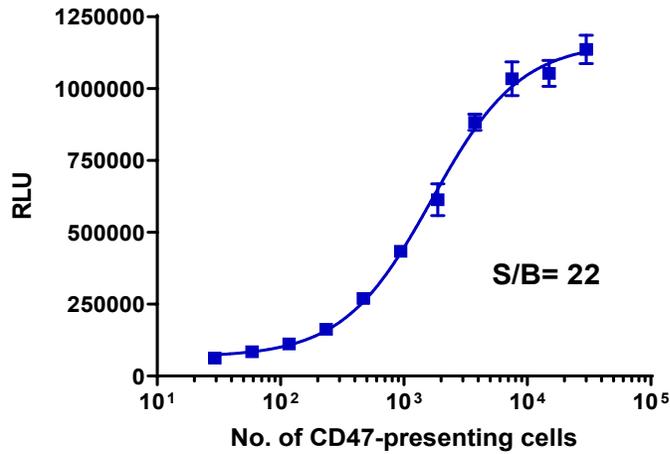
Assay quantifies ligand-induced recruitment of SHP-1 to ITIM motifs in C-terminal tail of SIRP α in response to phosphorylation

PathHunter® SIRPα (CD47) Signaling Assay

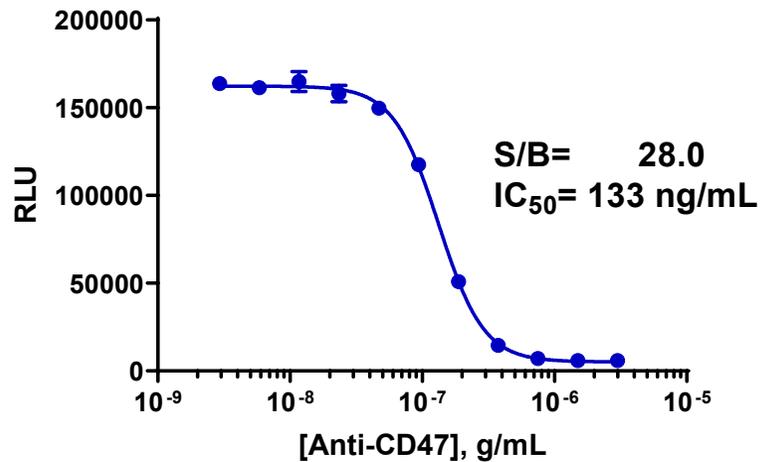
Co-culture model with stable surface expression of SIRPα and a stable functional response over 45+ passages

Cell-Presented Ligand

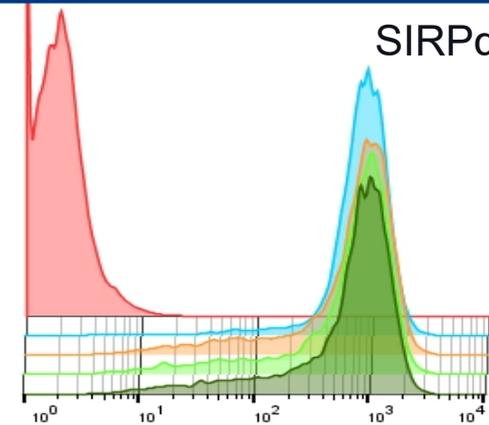
Agonist



Antagonist

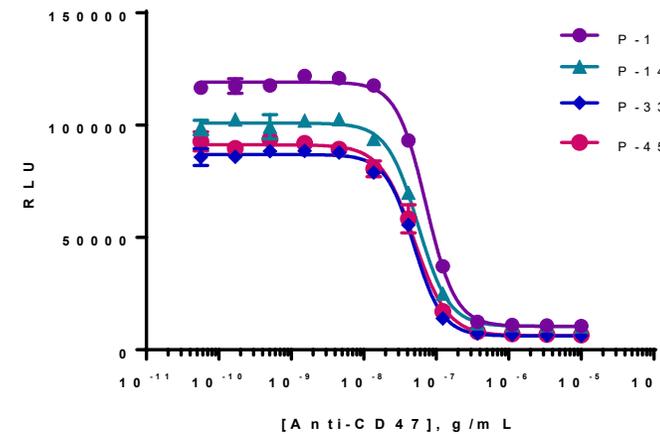


Stable SIRPα Surface Expression



SIRPα expression varies by <20% RSD over 45 passages

Stable Functional Response

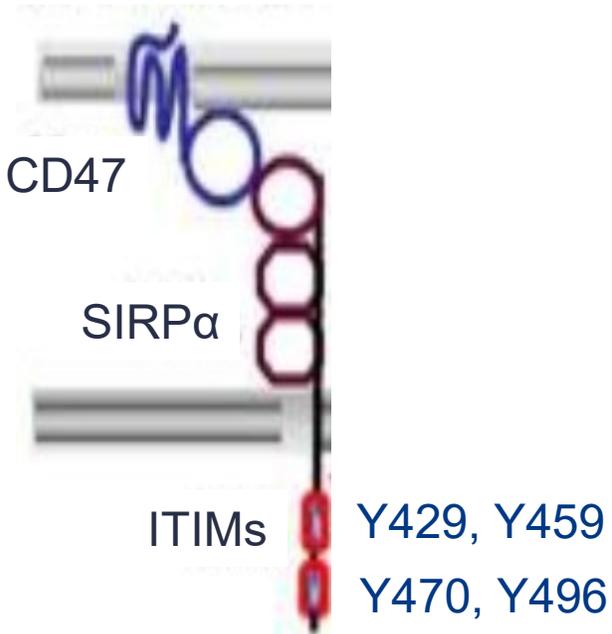


S/B : 17% RSD over 45 passages

IC₅₀ : <19% RSD over 45 passages

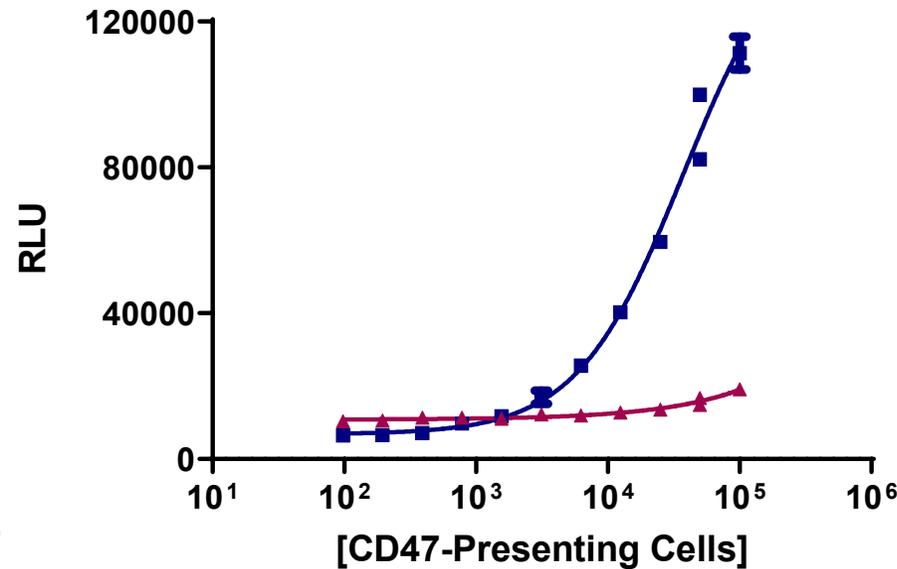
Mutation of SIRP α ITIM Motifs Disrupts CD47-Mediated Signaling

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



Agonist

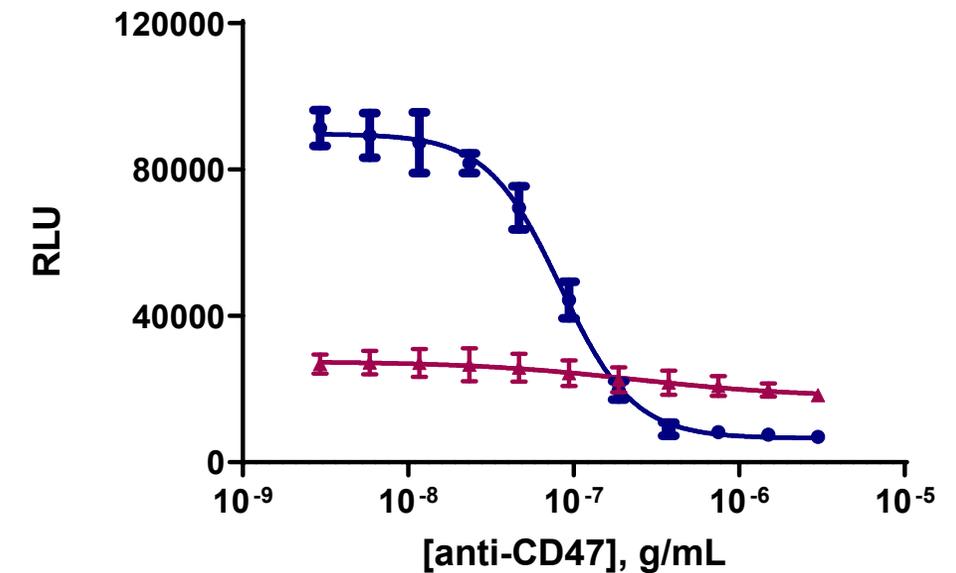
Jurkat SIRP α Signaling Assay



▲ SIRP α (Y429F, Y459F, Y470F, Y496F)
■ W/T SIRP α

Antagonist

Jurkat SIRP α Signaling Assay

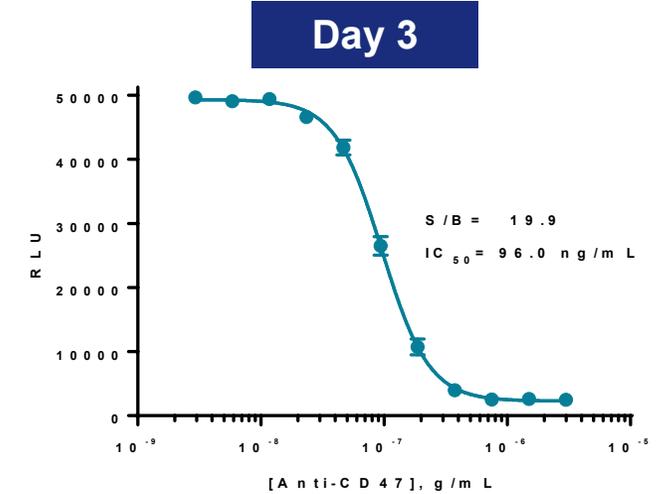
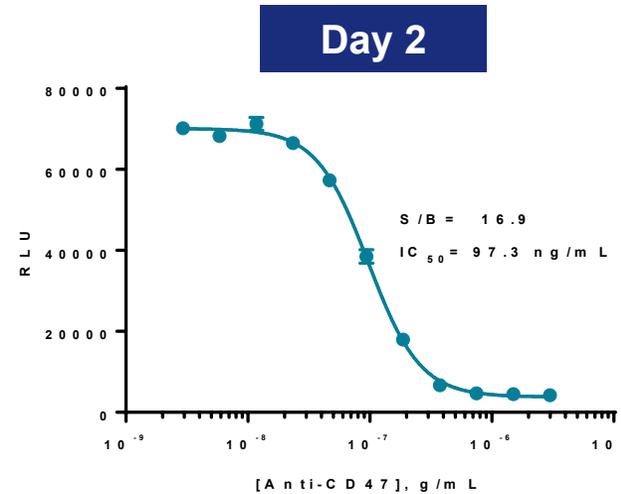
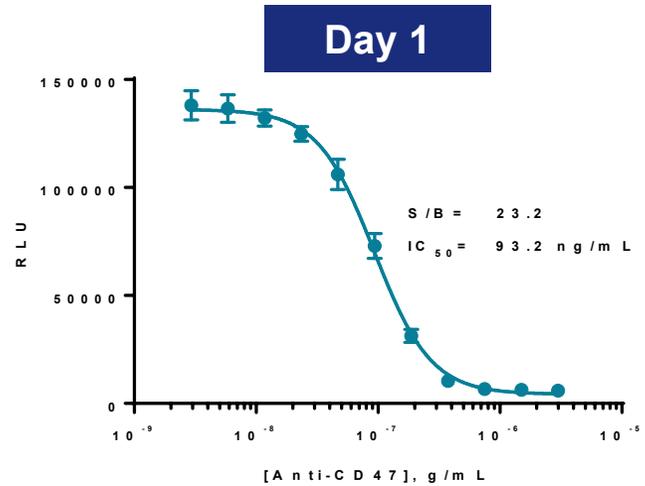


▲ SIRP α (Y429F, Y459F, Y470F, Y496F)
● W/T SIRP α

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 SIRP α 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%

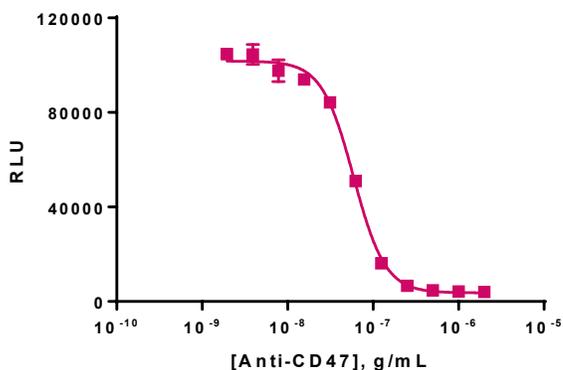
Development of RTU Assay Format for SIRP α Signaling Assay, with an Easy-to-Transfer Method

RTU (Bioassay) Method

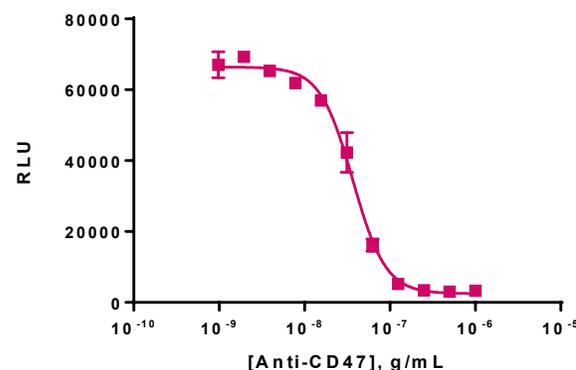


Total Assay Time:
~26 hours

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

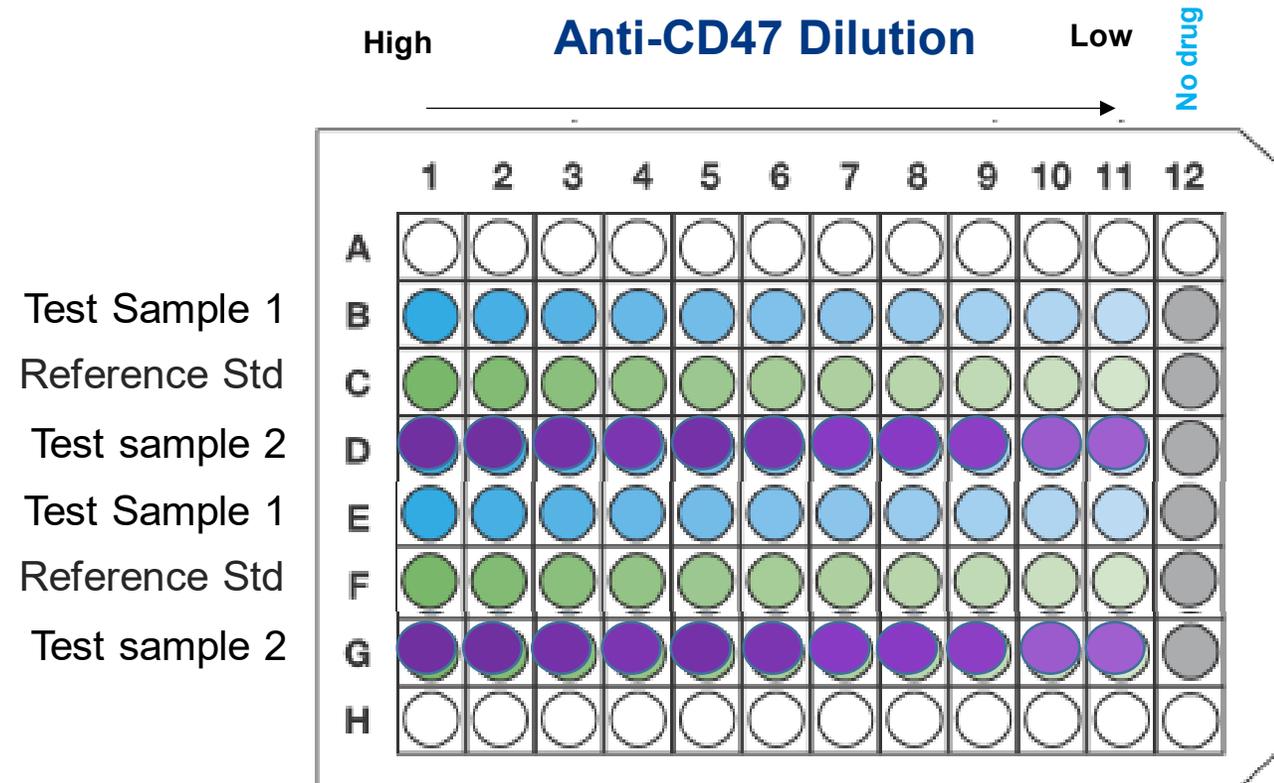
SIRP α Bioassay Qualification: Study Design

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 SIRP α bioassay

Study Design

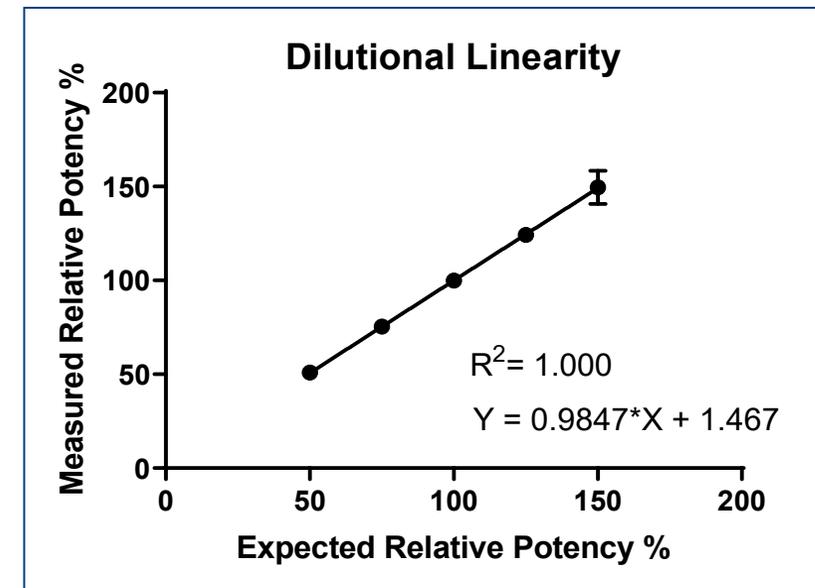
- Two analysts, multiple days
- 5 sample concentrations over a range of 50-150% (50%, 75%, 100%, 125% and 150%)
- Each concentration evaluated 3 times by each analyst over a minimum of 3 days
 - Each sample tested in duplicate wells per dose with interleaved plate layout
- Specificity and forced degradation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

Example plate layout



SIRP α Bioassay Qualification: Excellent Accuracy and Dilutional Linearity Over Range of 50-150%

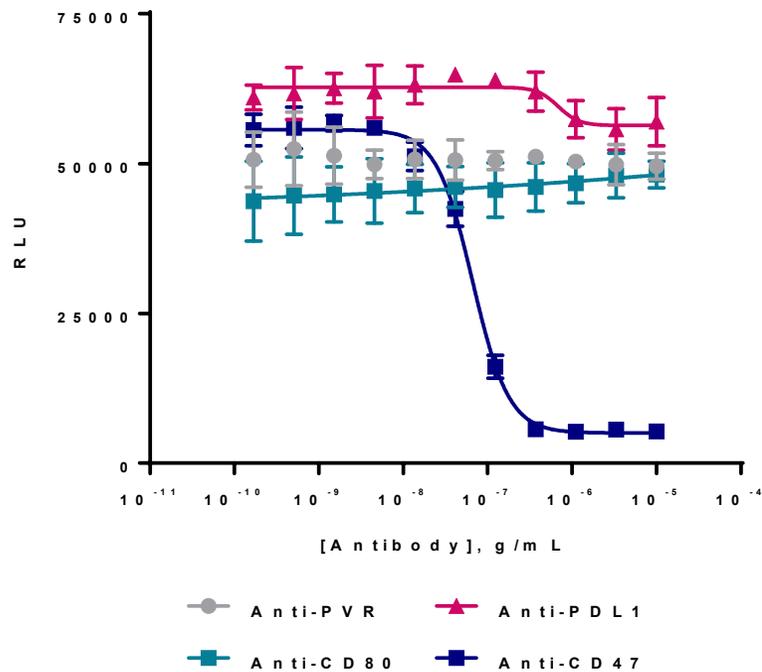
Expected RP (%)	Exp #	Analyst #	Measured RP (%)	Average RP (%)	% RSD	% Accuracy	Relative Bias, %
150	1	1	164	149.5	5.96	99.7	-0.3
	2	1	144				
	3	1	145				
	4	2	140				
	5	2	148				
	6	2	156				
125	1	1	123	124.2	3.16	99.4	-0.6
	2	1	125				
	3	1	124				
	4	2	119				
	5	2	123				
	6	2	131				
100	1	1	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				



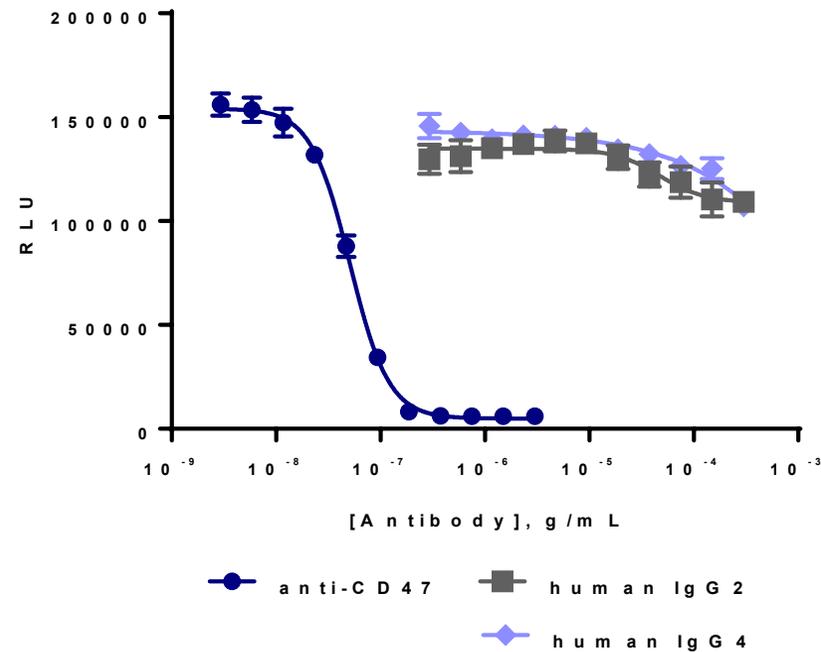
Accuracy	100.02%
Intermediate precision	6.5%
Relative Bias	1.6%
Dilutional Linearity	$R^2 = 1.000$
Range	50-150%

PathHunter SIRP α Signaling Assay: Excellent Specificity and Stability-Indicating Properties

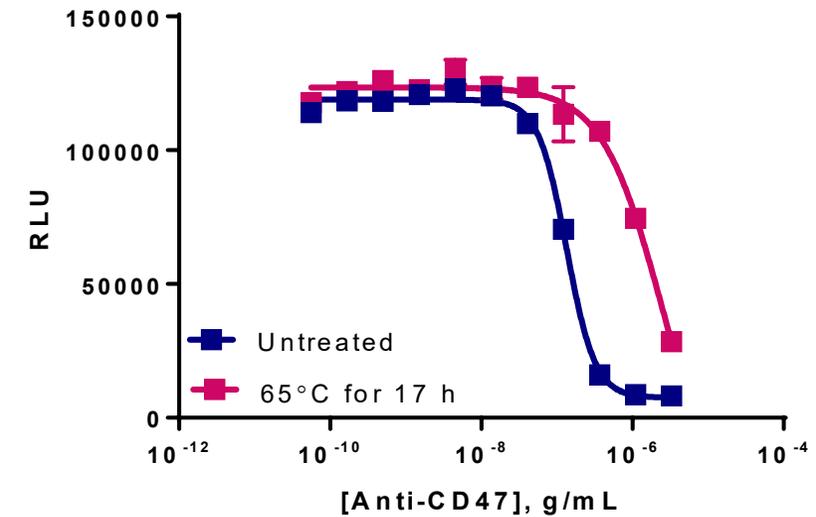
Assay Specificity



Assay Specificity

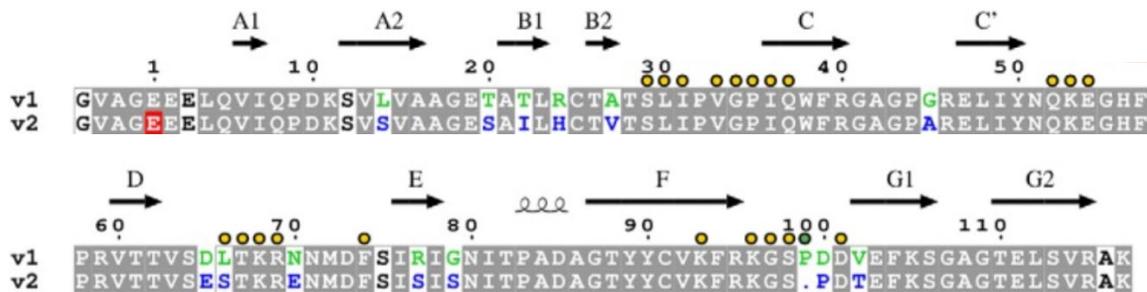


Stability-Indicating



Signaling Assays for Most Common SIRP α Variants: V1 and V2

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



Published by the American Society for Biochemistry and Molecular Biology

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

PMCID: PMC3974974

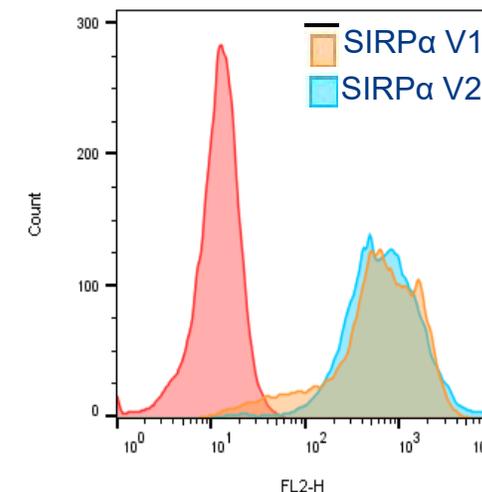
Published online 2014 Feb 18. doi: [10.1074/jbc.M114.550558](https://doi.org/10.1074/jbc.M114.550558)

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

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

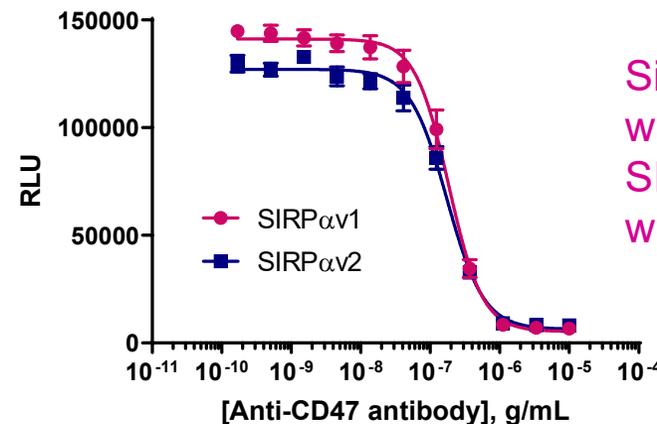
Deborah Hatherley, Susan M. Lea, Steven Johnson, and A. Neil Barclay¹

SIRP α Surface Expression



Comparable surface expression for SIRP α V1 and V2

SIRP α Functional Response



Similar assay window and IC₅₀ for SIRP α V1 and V2 with anti-CD47

PathHunter SIRP α Signaling Assay

Ready-to-Implement solutions for your development programs

First commercially-available SIRP α /CD47 cell-based assay

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

- Mechanistically-relevant endpoint (SHP1 recruitment) that robustly quantifies CD47-mediated SIRP α signaling
 - Assays available for both SIRP α V1 and SIRP α V2 variants
 - Suitable for anti-CD47 or SIRP α -targeted therapeutics
- Delivers highly specific response
- Expression of SIRP α 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 β Superfamily
- ADCC Assays
- ADCP Assays
- CDC Assays
- Target Cells
- Effector Cells

Technical Support: DRX_SupportUS@eurofinsUS.com