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The Eurofins Discovery PRODUCTS COMPANY

Accelerating Immunotherapy Drug Development Targeting Checkpoints with Robust MOA-Based Assays

Presented by Dr. Alexander Baumann

Head of European Business Development

Eurofins DiscoverX Products

Meet us at BOOTH #24

Eurofins DiscoverX – Global Leader in Cell-Based Assays for Screening, Profiling, Potency, & Lot Release Programs

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The Eurofins Discovery PRODUCTS COMPANY

From Discovery to Development to Clinic to Post-Market

20+ Years of Enabling Drug Discovery and Development Programs

San Francisco Bay Area, California

R&D & Manufacturing
San Francisco Bay Area, California (HQ)
St. Charles, Missouri
Poitiers, France

10+
Druggable target classes

1500+
Stable cell line and membrane preps

20+
Core patents

2000+
Publications across multiple applications

55+
Qualified & MOA- based Bioassays

Validated
>30 Billion Data Points
screened in assay services with same assays

3 Certified CRO Partners
Scientific training to enable global CROs

ICH Based Bioassay Qualification
Facilitate downstream validation studies

Dedicated Scientific Support
Experienced team providing scientific support

20+ Successful Assay Transfers
At clients/affiliated CRO sites

50+ Global Programs
For potency, stability and NAb testing

MOA = Mechanism-of-Action; ICH = International Council for Harmonization; CRO = Contract Research Organizations; NAb = Neutralizing Antibody

Enzyme Fragment Complementation (EFC) Technology

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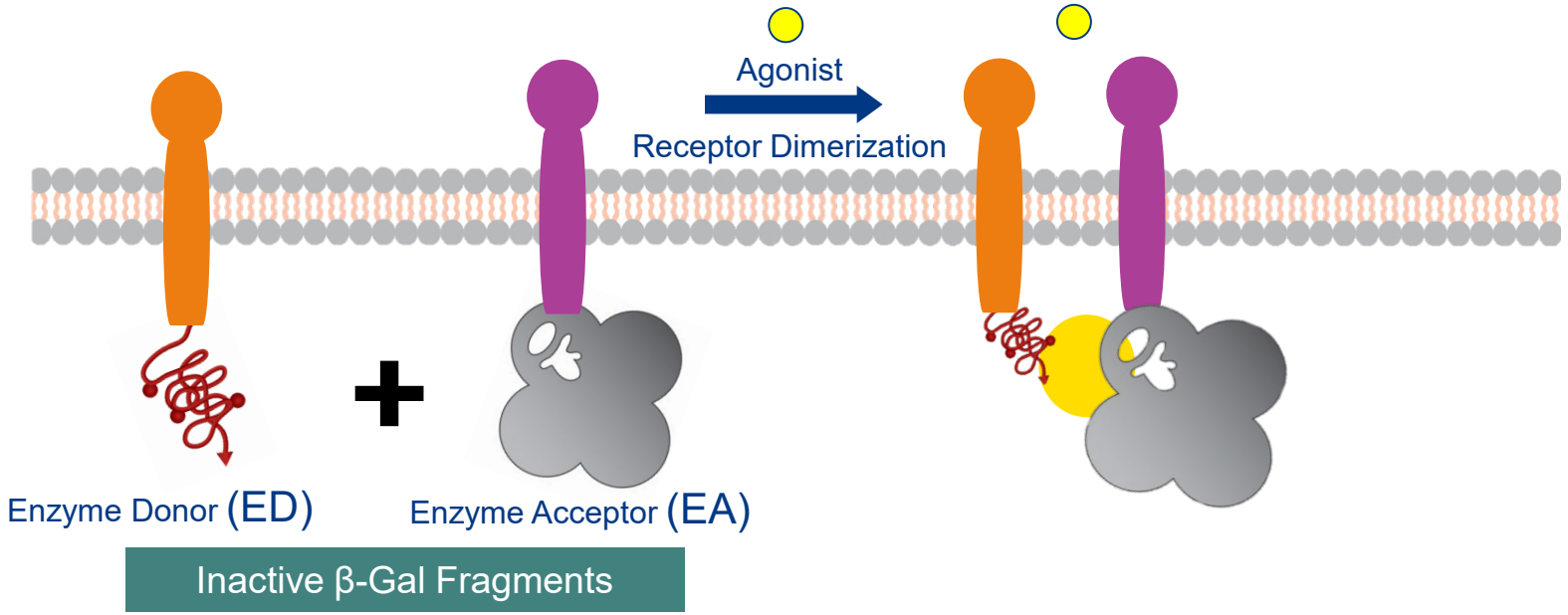
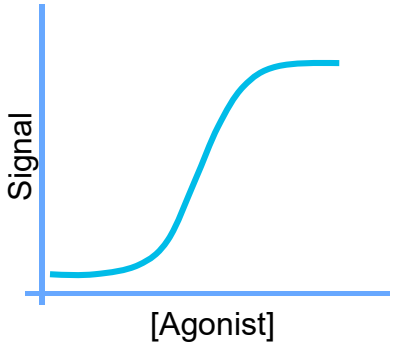
Assay Design: e.g. Receptor Dimerization Assay (MOA for RTKs and Interleukins)





Homogenous

- Add-and-read assay format
- No washing steps
- No centrifugation step
- No shaking or filtration

Robust

- Enzymatically-amplified Assay
- Large signal-to-background
- High precision and reproducibility

Qualified and Validated

- Optimized for hundreds of targets
- Screened billions of data-points
- >2K peer-reviews publications

Transferrable

- Assay protocol is similar across platform
- Detailed user manuals
- Assays routinely transferred between multiple sites

Enzyme Fragment Complementation (EFC)

Lego®'s for Assay Developers!

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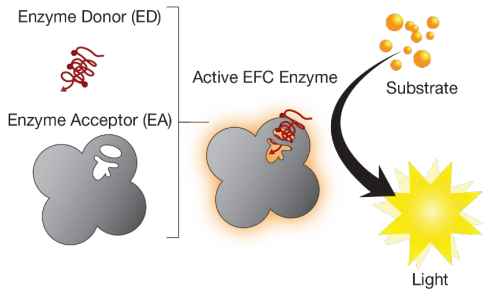
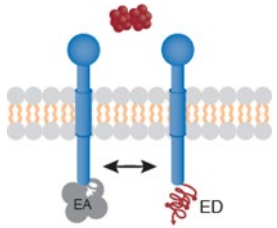
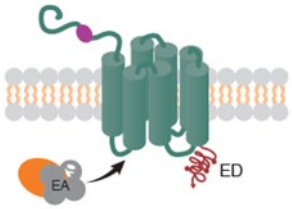
Split β-galactosidase reporter system can be engineered to generate target-specific, homogeneous cell-based assays for immuno-oncology therapeutics



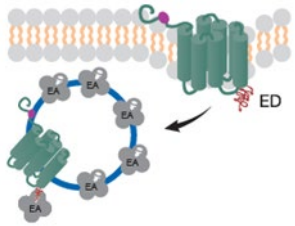
Receptor Dimerization



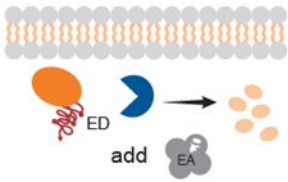
Protein/Protein Interactions



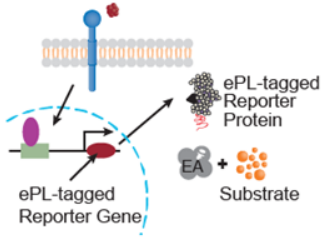
Internalization & Trafficking



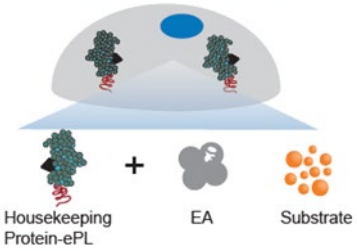
Quantification & Degradation



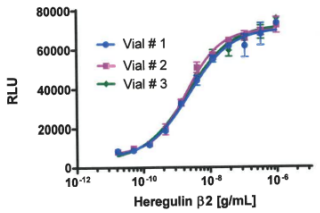
Downstream Response Evaluation



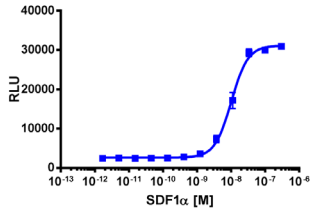
Quantify Cytotoxicity



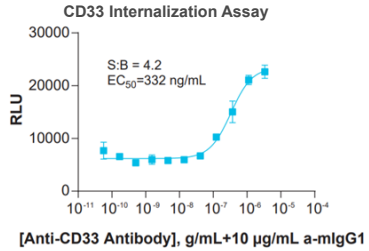
ErbB2/ErbB3 Dimerization Assay



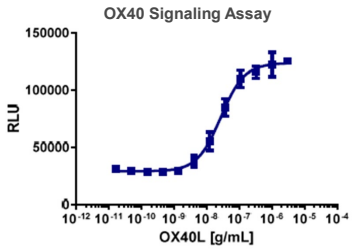
CXCR7 β-Arrestin Recruitement Assay



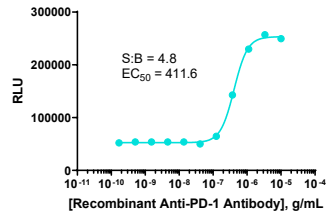
CD33 Internalization Assay



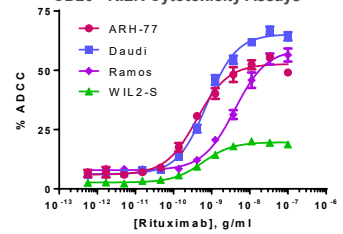
OX40 Signaling Assay



Jurkat PD-1 NFAT Reporter Assay



CD20+ KILR Cytotoxicity Assays



PathHunter® Checkpoint Receptors Assays
Industry's Largest Menu of Stable Cell Lines and Bioassays

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Functional Cell-Based Assays

Immunoglobulin Superfamily (IgSF)		TNFR Superfamily (TNFRSF)
SIRPα	CD47	CD137
ICOS		CD40
CD28		OX40
CD200R	CD200	CD27
PD1 (SHP1)	PD-L1	
PD1 (SHP2)	PD-L2	
BTLA	HVEM	
CTLA4	CD86	
mPD1	mPD-L1	
mCTLA4	mCD80	
Signaling Cell Lines	Ligand Cell Lines	

Tools for Testing Agonist Antibodies

Clustering Cell Lines

FcγRIa | FcγRIIa | FcγRIIb

Early Access Cell Lines

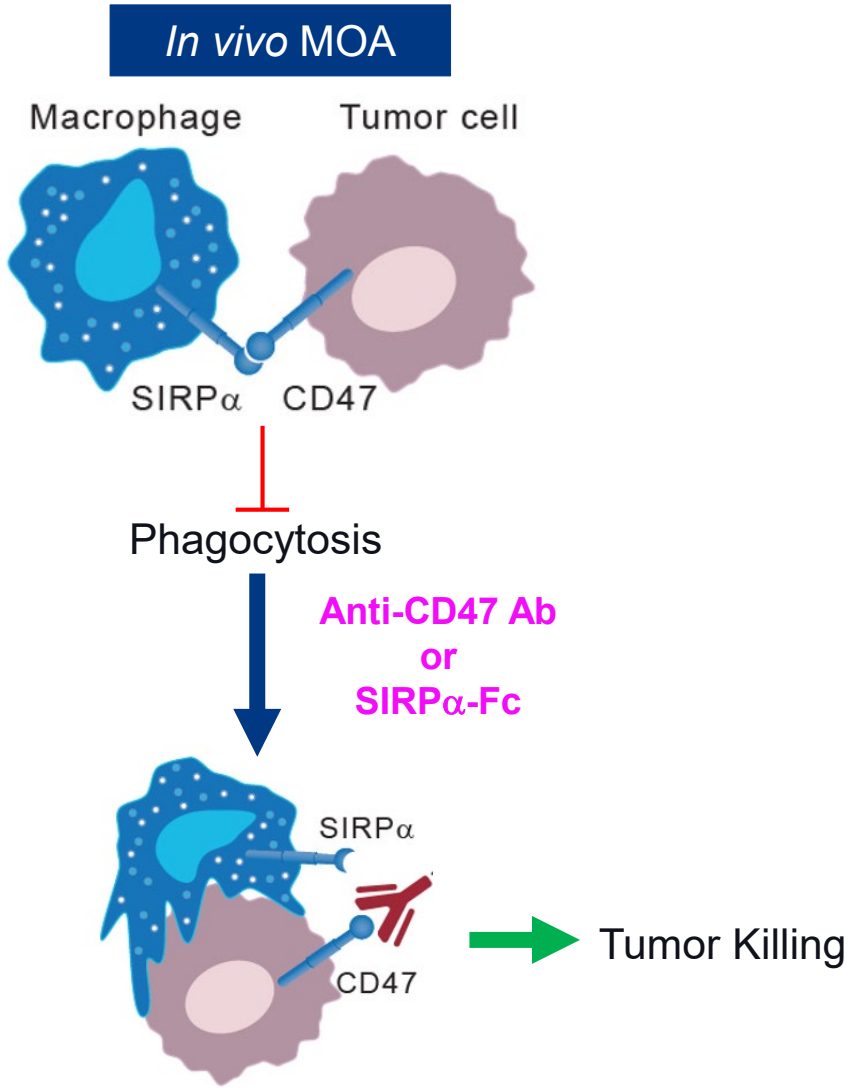
Binding Assays for Bi-specifics

PD-1/LAG3	PD-1/PD-L1
PD-1/CTLA4	PD-L1/CTLA4
PD-1/TIGIT	PD-L1/TIM3
PD-1/CEACAM1	TIM3/CEACAM1
PD-1/CD28	TIGIT/LAG3
mPD-1/mLAG3	mPD-1/mTIGIT
mPD-1/mCTLA4	

Case Study: PathHunter® SIRPα Signaling Bioassay Development

For Therapeutics Targeting CD47|SIRPα Signaling Axis

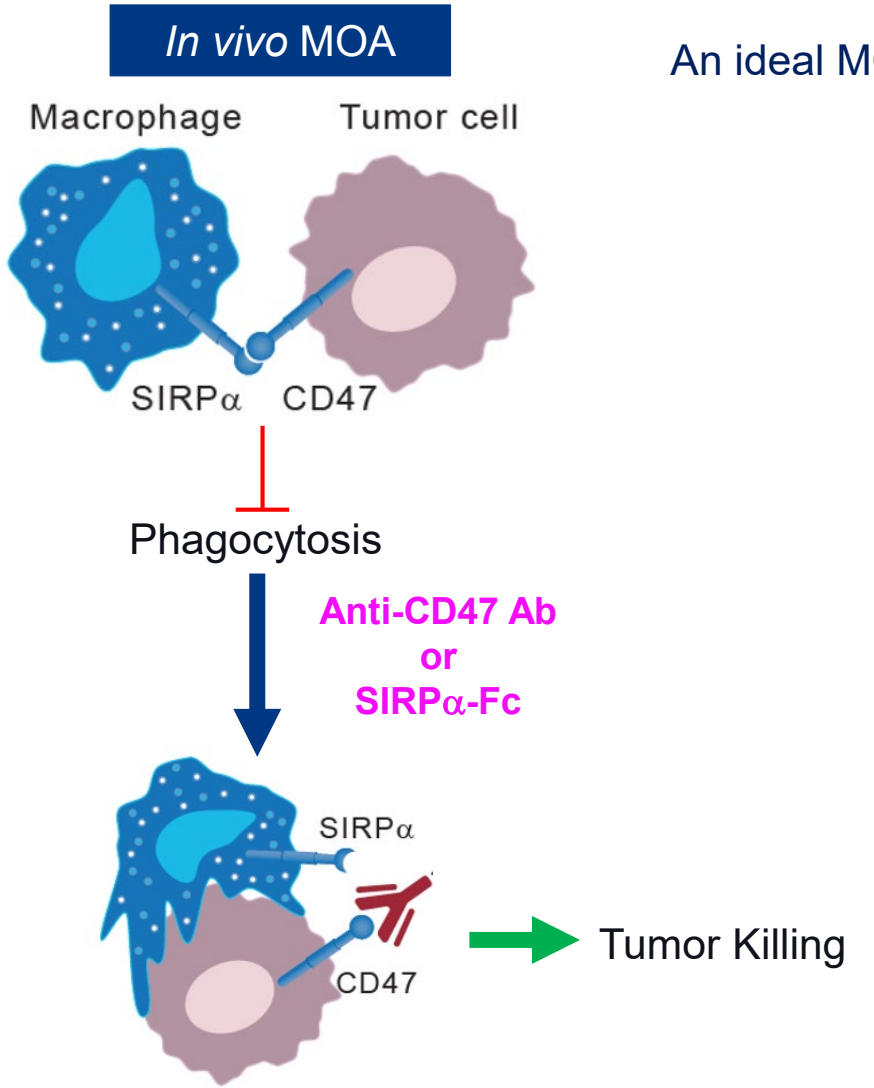
The SIRPα / CD47 Axis - Innate Immune Checkpoint



The SIRPα / CD47 Axis

- **SIRPα:** inhibitory receptor expressed on macrophages and dendritic cells that promotes phagocytosis of foreign objects
- **CD47**: ligand for SIRPα, expressed on nearly all cells, but significantly up-regulated in many tumor types
- **Blocking** the CD47 / SIRPα axis promotes tumor killing

The SIRPα / CD47 Axis - Innate Immune Checkpoint



An ideal MOA-reflective cell-based assay would quantify phagocytosis endpoint

Challenges with Phagocytosis Assays

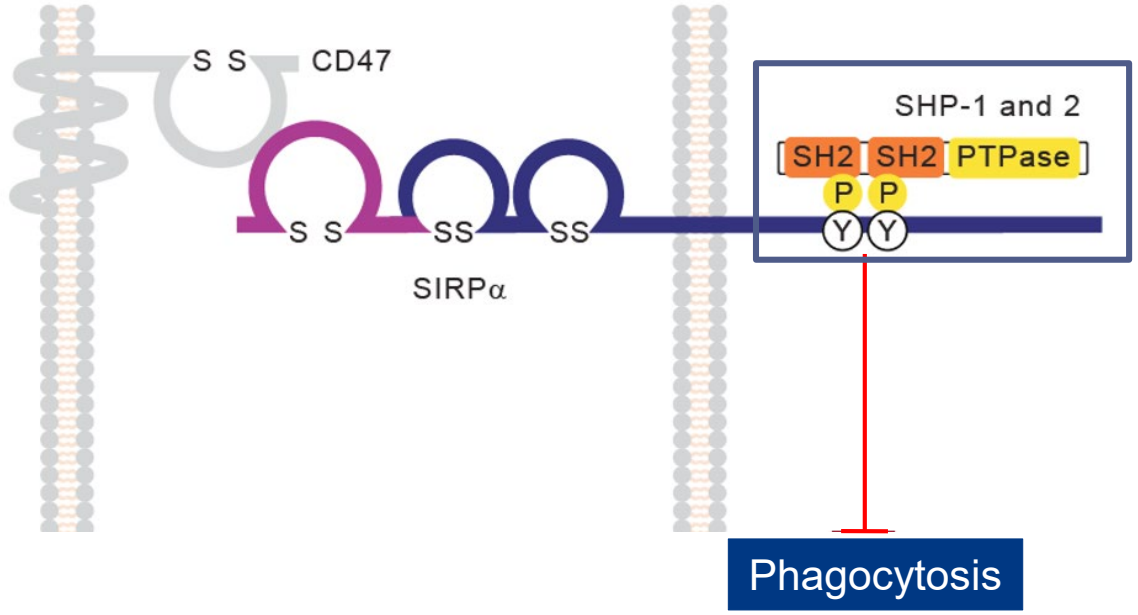
- Typically evaluate co-localization of macrophage and target cells using flow cytometry or high content imaging
- Require use of human macrophages as effector cells
  - Macrophage cell lines not reliable as effectors
  - Differentiation from primary monocytes takes 7 days
  - Activated M1 macrophages difficult to lift (30’ to 60’)
- A more ‘QC-friendly’ MOA reflective assay is required

PathHunter® SIRPα Signaling Assay
Assay Concept

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

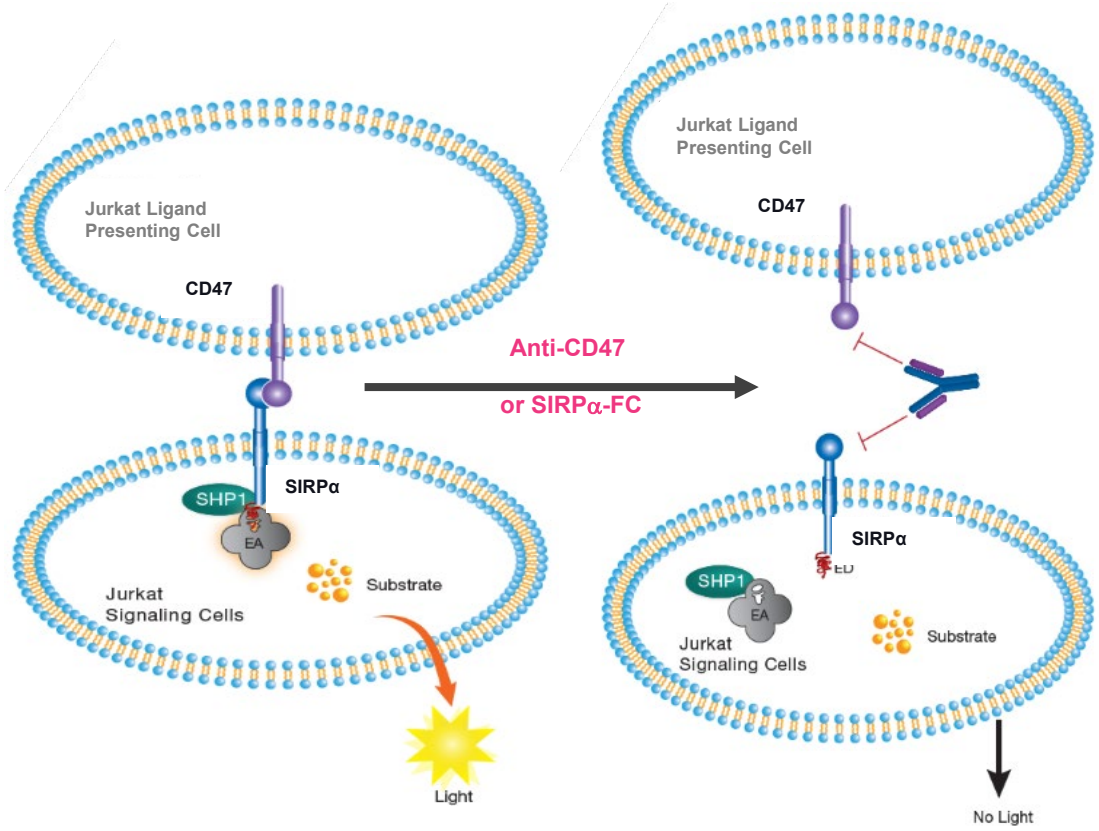
Molecular MOA

Tumor Cell **Macrophage**



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

Co-Culture SHP Recruitment Model

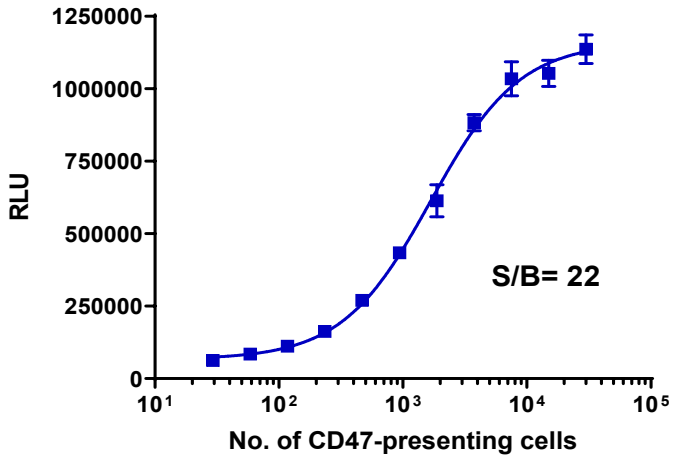


PathHunter® SIRPα (CD47) Signaling Assay

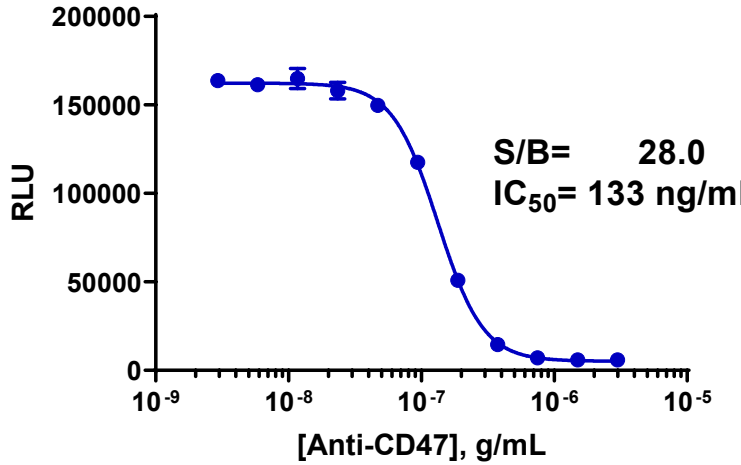
Co-culture model with stable surface expression of SIRPα and a stable functional response over 45+ passages

Cell-Presented Ligand

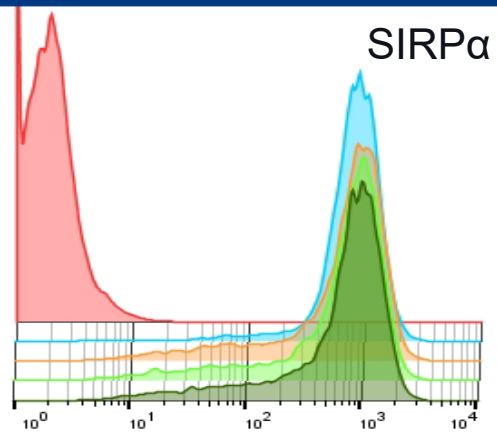
Agonist



Antagonist

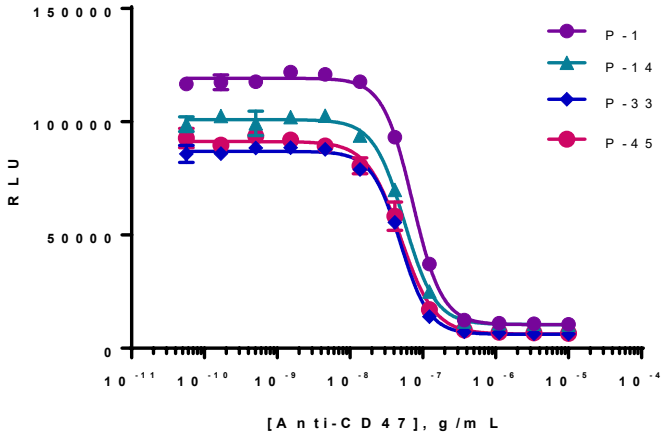


Stable SIRPα Surface Expression



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

PathHunter® SIRPα (CD47) Signaling Assay
Excellent Specificity and Stability-Indicating Properties

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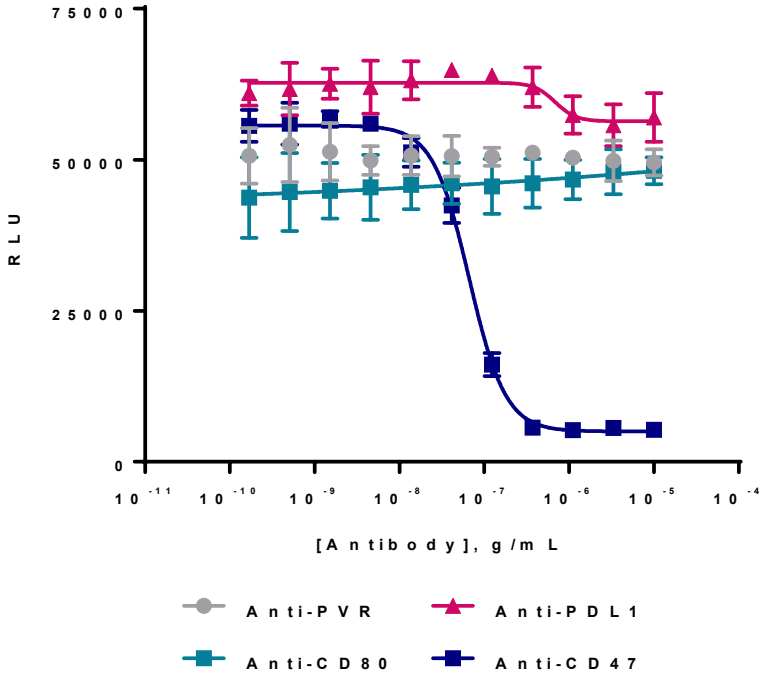
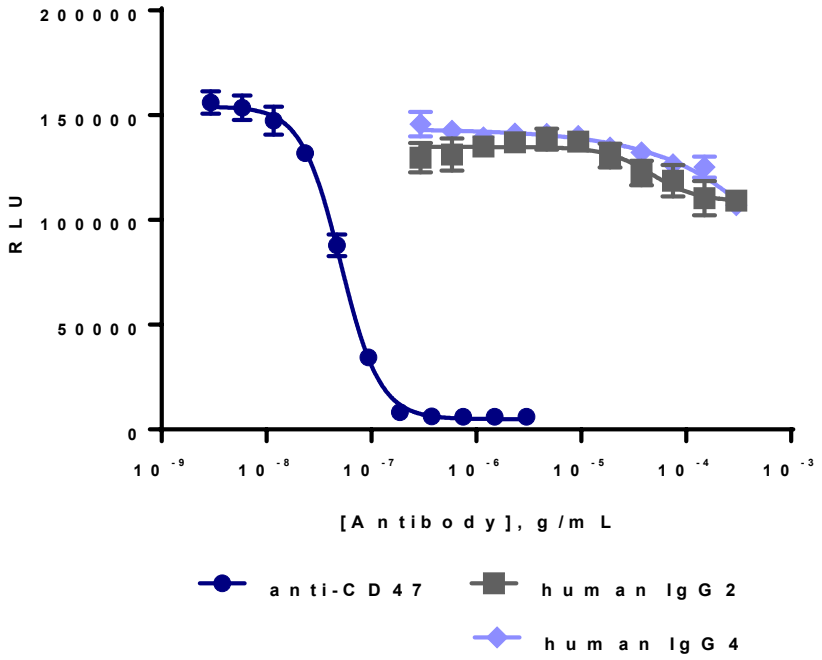
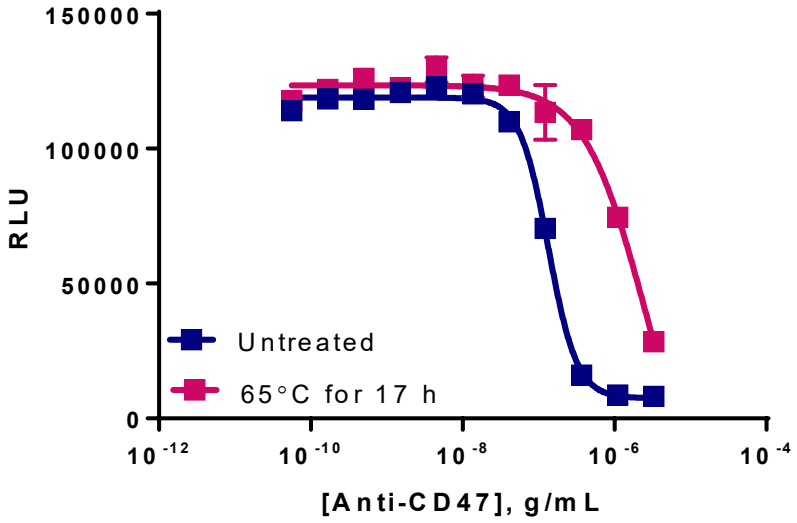
Assay Specificity



Assay Specificity



Stability-Indicating



Development of RTU Assay Format for SIRPα Signaling Assay

Easy-to-Transfer Method

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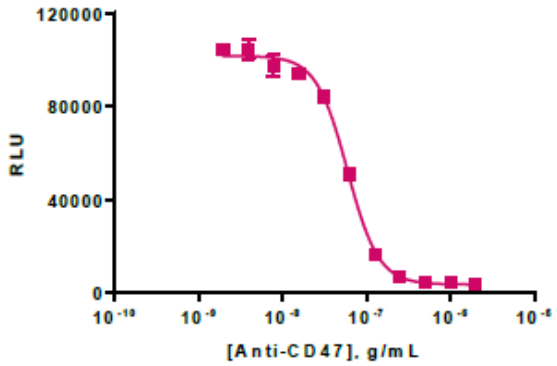
RTU (Bioassay) Method

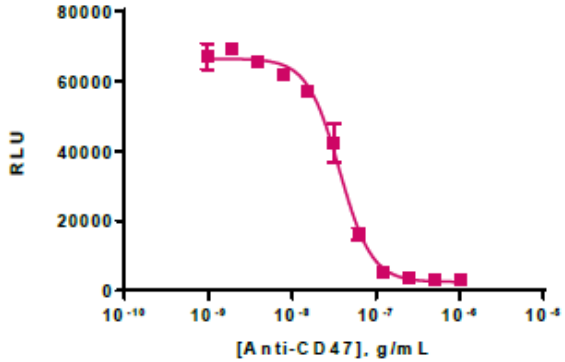
Thaw and add CD47 and SIRPα frozen cells (1 vial each) to assay plate → Prepare antibody dilutions and add to assay plate → 24h → Add Detection Reagent to assay plate → 1h → Read signal on plate reader

Total Assay Time:
~26 hours

Continuous Culture



Cryopreserved (RTU)

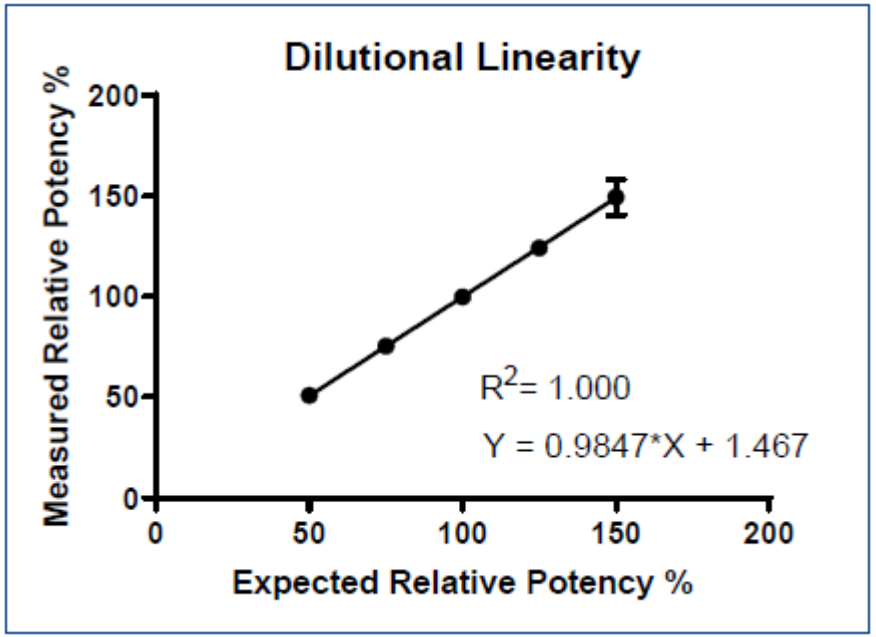


Comparable Performance to Continuous Culture Format

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

SIRPα Bioassay Qualification

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				



Dilutional Linearity: R^2 =1.000

Accuracy: 100.02%

Intermediate Precision: 6.5%

SIRPα Bioassay is highly accurate, precise and linear!

Examples of Qualified Bioassays for Precise Potency Determination for Therapeutic Drugs

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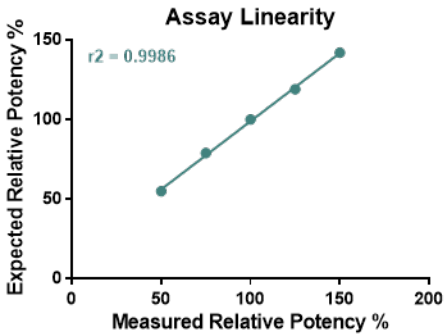
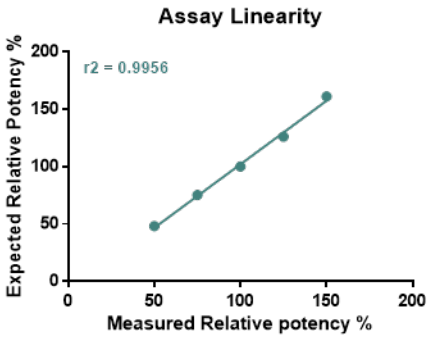
- Performance Qualification
  - ✓ Relative Accuracy
  - ✓ Specificity
  - ✓ Intermediate Precision
  - ✓ Range

PD-1
Pembrolizumab (Keytruda)



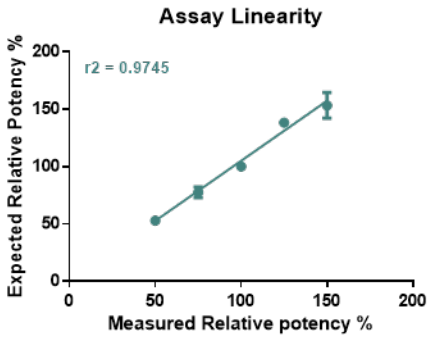
Pembrolizumab
Accuracy = 99.7%
Precision = 4.8%

EGFR/ERBB2
Panitumumab (Vectibix)



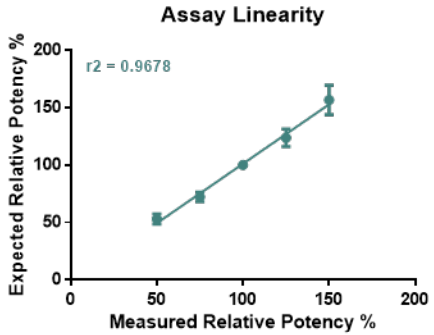
Panitumumab
Accuracy = 103.4%
Precision = 4.6%

IL6R/IL6ST
Tocilizumab (Actemra)



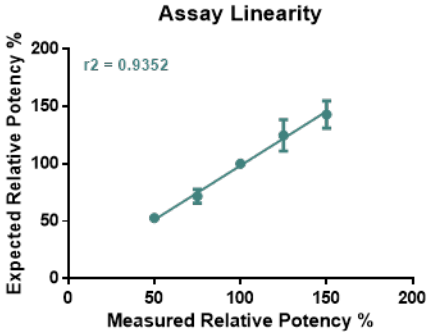
Tocilizumab
Accuracy = 105.5%
Precision = 4.7%

KDR/KDR
Ranibizumab (Lucentis)



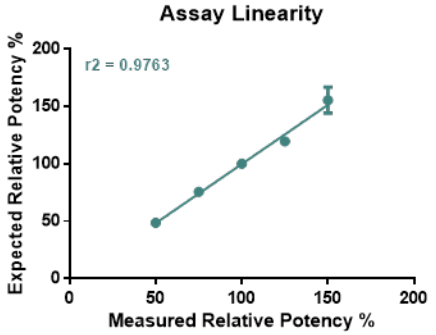
Ranibizumab
Accuracy = 100.5%
Precision = 5.4%

PTHR1
Teriparatide (Forteo)



Teriparatide
Accuracy = 100.4%
Precision = 9.9%

GLP1R
Exenatide (Byetta)



Exenatide
Accuracy = 100.8%
Precision = 5.2%

A central requirement for bioassays is the ability to accurately report drug potency using a functionally relevant readout

Eurofins DiscoverX bioassays are qualified with excellent linear potency as demonstrated with clinically relevant drugs.

Accelerating Implementation Phase for QC Lot Release with Qualified Bioassays

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Typical Bioassay Development Timeline

Time to Market >12 months

Characterize Cell Line	Assay Optimization	Testing Innovator Drug	Assay Qualification	Lot/s Manufacture	Method Transfer (Client/CRO)	Assay Validation	QC Lot Release
• Choose assay format that best reflects MOA of drug	• Optimize assay parameters (thaw-and-use cells)	• Refine conditions with innovator drug	• Establish robustness, precision, accuracy, and linearity of assay	• Generate lots for bioassay validation	• Method review • On-site transfer runs	• ICH Validation (GMP)	

Eurofins DiscoverX Bioassay Development Timeline

Time to market <3 months

Characterize Cell Line	Assay Optimization	Testing Innovator Drug	Assay Qualification	Lot/s Manufacture	Method Transfer (Client/CRO)	Assay Validation	QC Lot Release
☑ **DONE** Cell Line established	☑ **DONE** Bioassay developed	☑ **DONE** Bioassay optimized with innovator drug	☑ **DONE** Qualification complete	☑ **DONE** Lots available for immediate use	• Method review • On-site transfer runs	• ICH Validation (GMP)	

Eurofins DiscoverX Ready-To-Plate Bioassay Kits Save 9 Months of Assay Development Time

Thank You and Please Join Us at Booth #24

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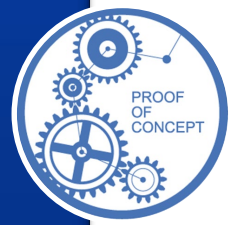
Options to suit your program needs

Purchase Membranes, Cell Lines or Ready to use Cells/Kits



Proof-of-Concept (Feasibility Study) or Custom Assay Development

Cell Line Rental (3-months Block)

- Qualified Bioassays
- Cell Lines
- Custom Assay Development
- Analytical Cell Banks
- GPCRs
- Checkpoint Receptors
- Cytokine Receptors
- Kinases
- Signaling Pathways
- TGFβ Superfamily
- ADCC Assays
- ADCP Assays
- CDC Assays
- Target Cells
- Effector Cells

For more information see discoverx.com/checkpoint or email Alexanderbaumann@eurofins.com (EU contact) or heatherzanetakos@eurofins.com (US contact).