DiscoverX

Advancing Therapeutics Targeting Cytokines with Functional Cell-based Assays

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Cytokines Are Key Mediators of Inflammation

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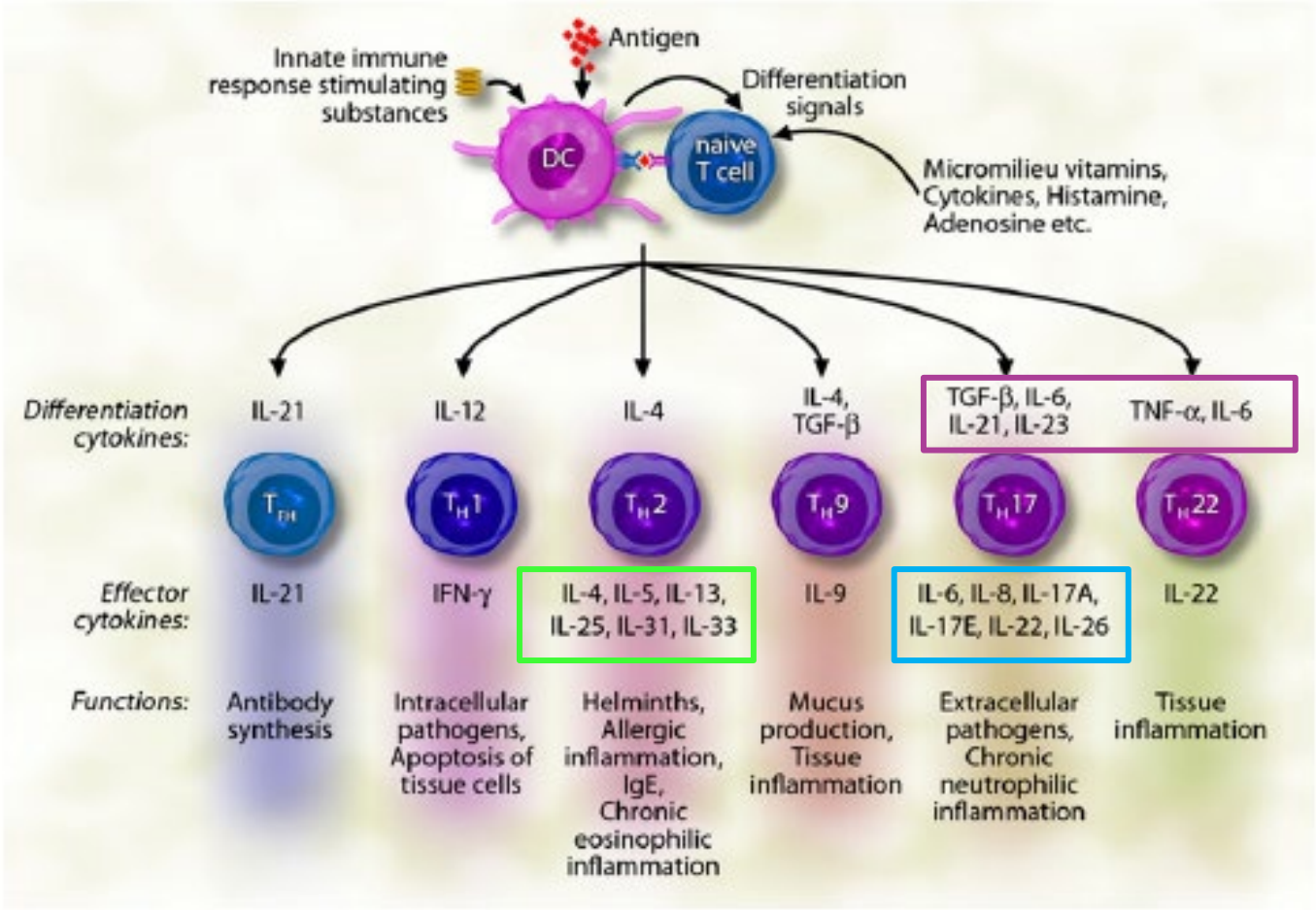
Cytokines produced in specific activated T cell populations are key mediators of inflammation and therapeutics



Approved Drugs Targeting Cytokines

Dupixent (Dupilumab)

Actemra (Tocilizumab)
Cosentyx (Secukinumab)

Humira (Adalimumab)
Stelara (Ustekinumab)
Tremfya (Guselkumab)
Skyrizi (Rizankizumab)

And others in clinical development…

Source: Akdis *et al. J Allergy Clin Immunol,* Vol 127. All registered trademarks are the property of their respective owners. IL = interleukin.

Top Selling Therapeutics Targeted Cytokines

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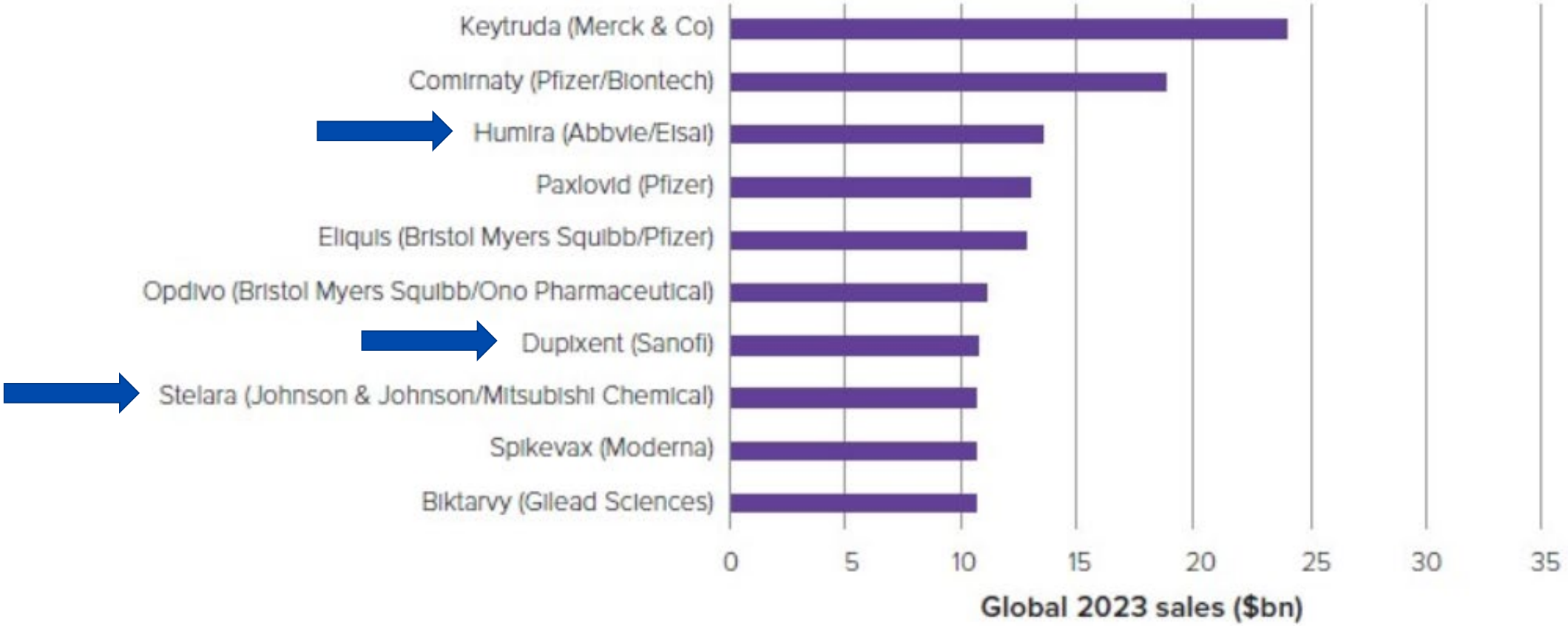
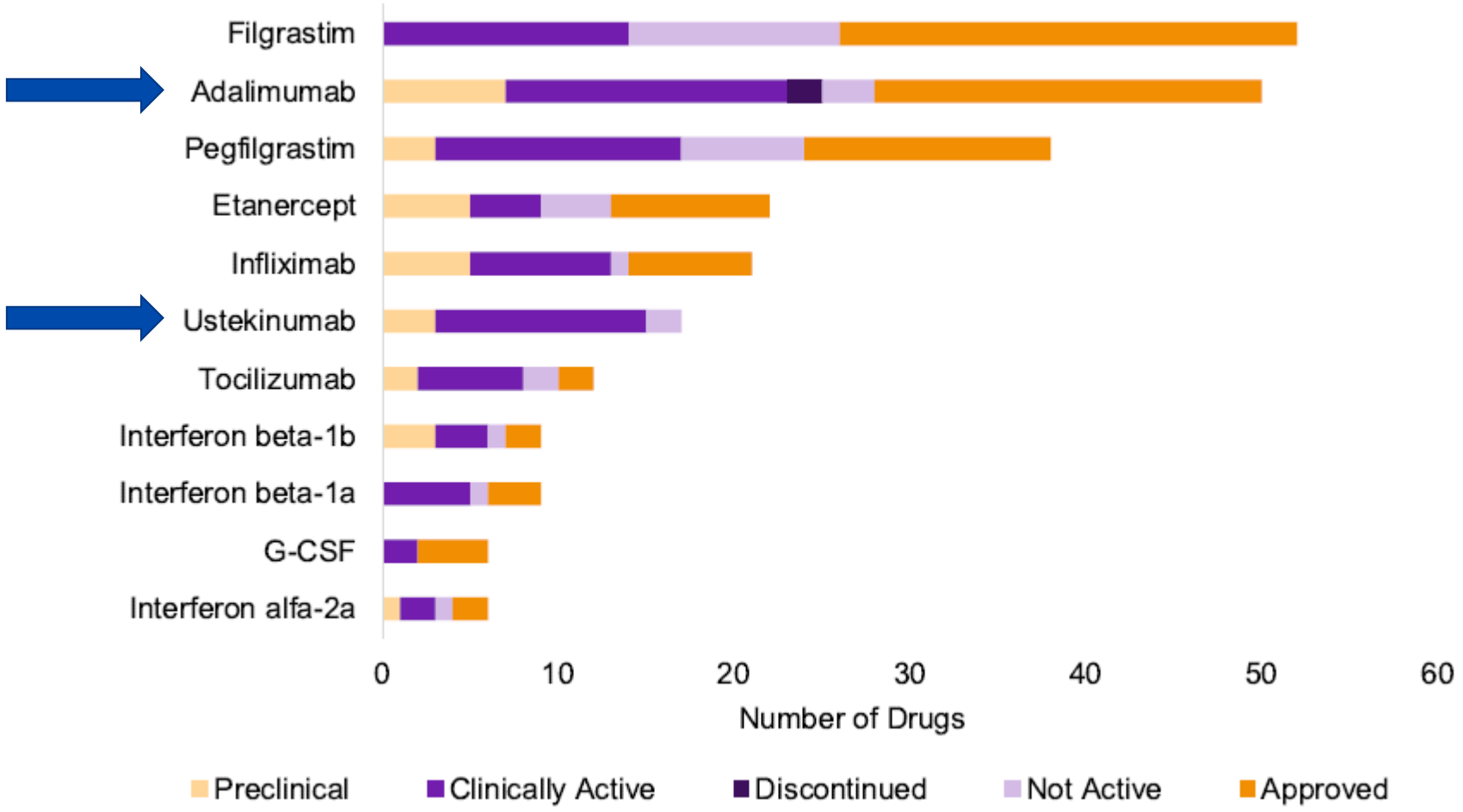


Source: Evaluate Vantage: The biggest-selling drugs of 2023. All registered trademarks are the property of their respective owners.

Cytokine Biosimilars Are Among the Top Selling Drugs



Source: Beacon Cytokine Webinar, 2023

Structural Diversity of Interleukin Receptor Families

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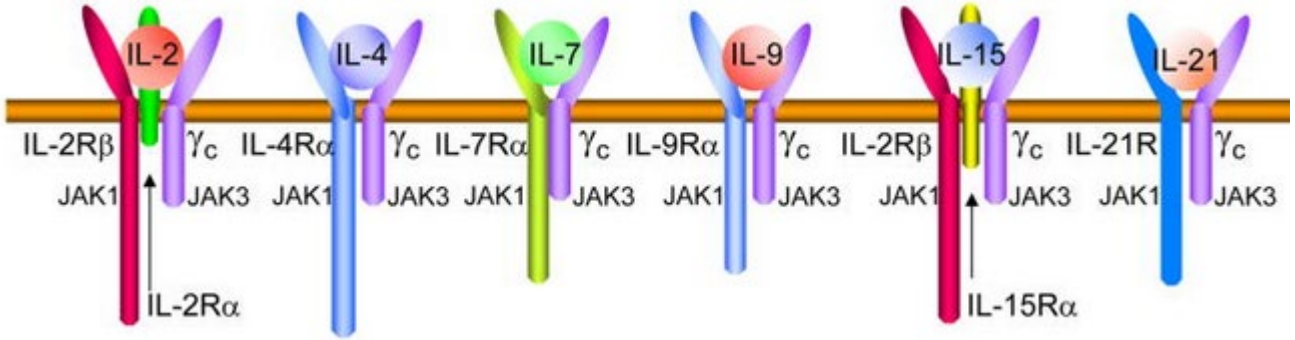
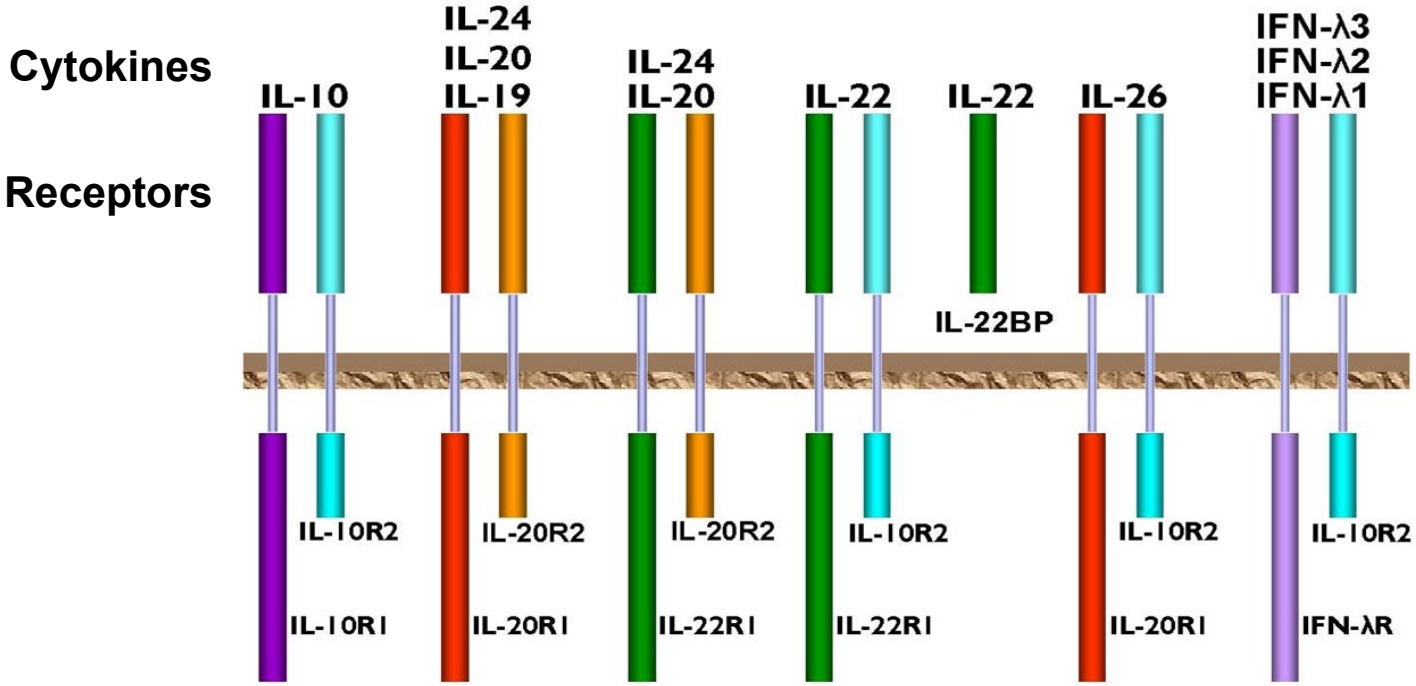
IL-2 Family

One receptor, multiple partners



IL-10 Family

Multiple receptors heterodimerize with multiple partners



Sources: Top image - Liao *et al.* (2011) *Curr Opin Immun* 23(5): 598-604. Lower image - Zdanov A. (2010) Cytokine Growth Factor Rev. 21(5): 325-30.

Cytokine Diversity

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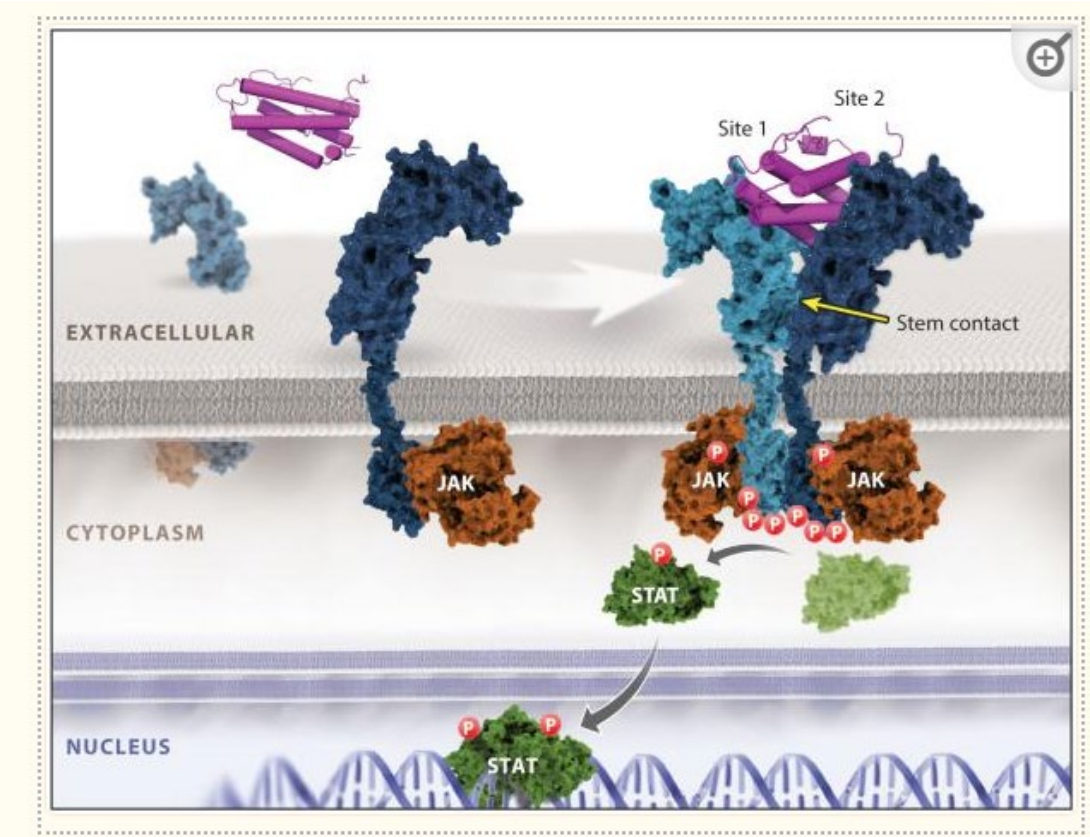
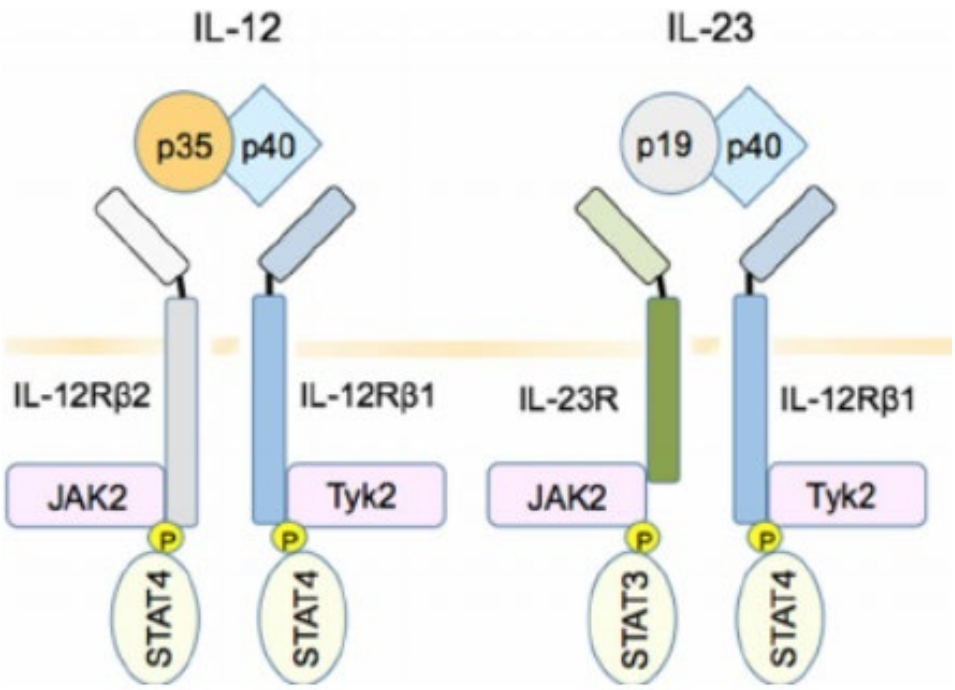
Cytokines are comprised of homomeric or heterodimeric subunits that bind to different receptor subunit combinations

Homomeric Ligands (e.g. IL-2, IL-4)



Heterodimeric Ligands (e.g. IL-12, IL-23)



Source (left image): Spangler *et al.* 2015. *Ann Rev Immunol* 33: 139-167.
Source (right image): Choi et al. 2015. *Clin Rev Allergy Immunol* 49(3):327-32

Eurofins DiscoverX® Large Menu Cell-Based Assays for Discovery, Potency, & NAb Assay Development

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>1,500+ Cell lines and membrane preps to support bioassay development for major drug target classes

289
Opioid
Glucagon
GABA
Neuropeptide
Chemokine
GPCRs

55
IGF1R
Tie2
ErbB1
ErbB2/B3
VEGFR
RTKs

30+
NRF2
TGFβ
IκB
SREBP
PI3
Pathway

8
NFkB
STAT3
STAT5
NFAT
Signaling Reporter

33
SIRPα
PD-1
CTLA-4
BTLA
OX40
Checkpoints

54
ADCC
ADCP
T-cell Redirection
CDC
Effector Cells
Cytotoxicity

19
ERa
PPARa
FXR
RARa
AR
NHRs

IL-1β
IL-6
IL-17
TNFα
GM-CSF
IL-2
Cytokine Storm
CCL3
G-CSF
CCL2
CXCL10
IL-10
IL-8
Cytokines & Interleukins

NAb = neutralizing antibody

Eurofins DiscoverX® Enzyme Fragment Complementation (EFC) Technology

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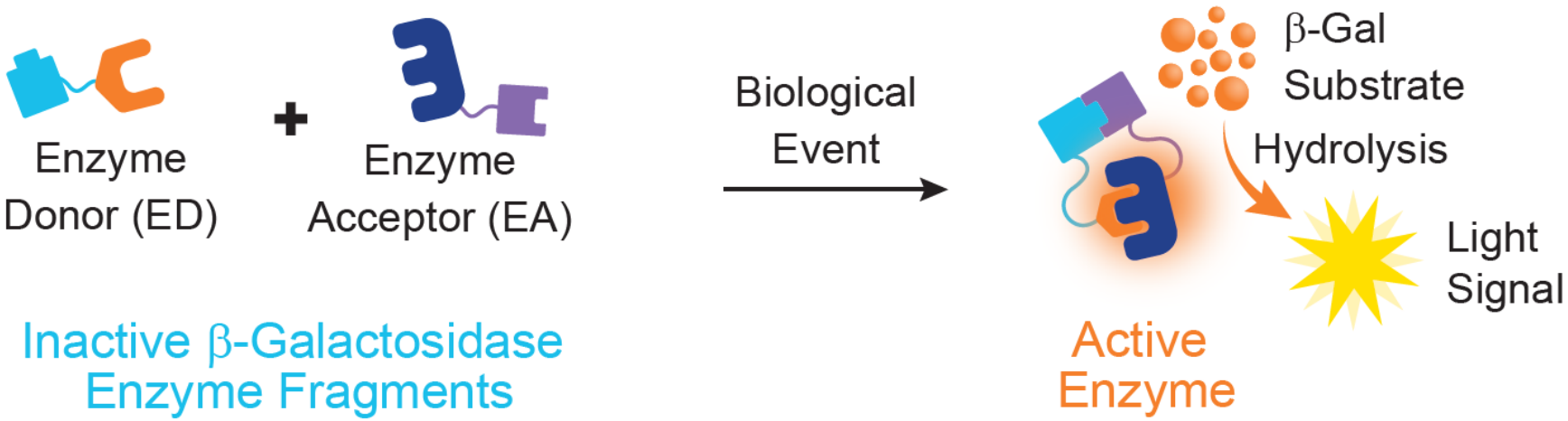
Enabling technologies with a flexible platform based on a split β-galactosidase enzyme



Homogenous	Robust	Qualified and Validated	Easy to Transfer
• Add-and-read assay format • No washing steps • No centrifugation step • No shaking or filtration	• Large signal-to-background • Gain of signal assay design • High assay accuracy, precision and reproducibility	• ICH-based Qualification • Validated at GMP CRO • Screened billions of data-points	• Assay protocol is similar across platform • Detailed user manuals • Method transfer support

ICH = International Council of Harmonization; GMP = Good Manufacturing Practices; CRO = Contract Research Organization

Receptor Dimerization Assay Design

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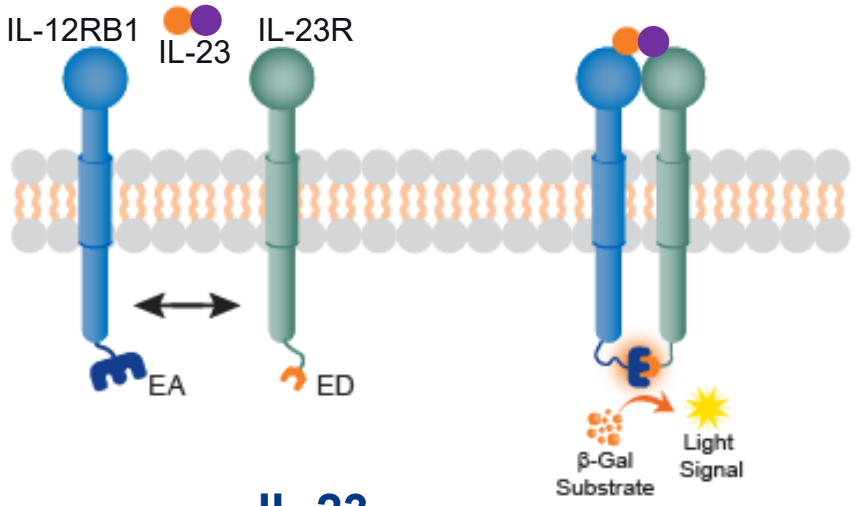
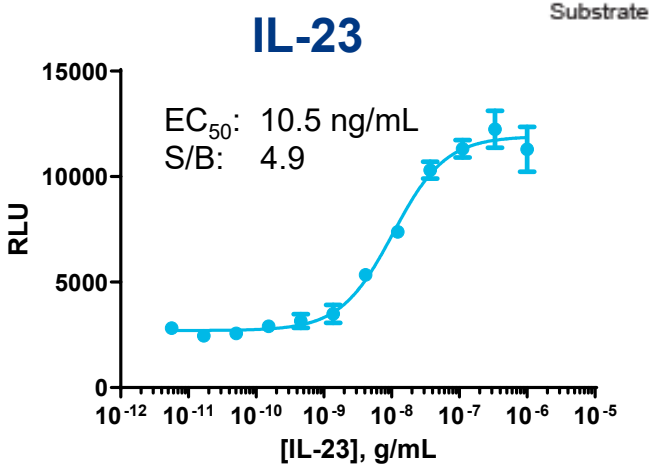
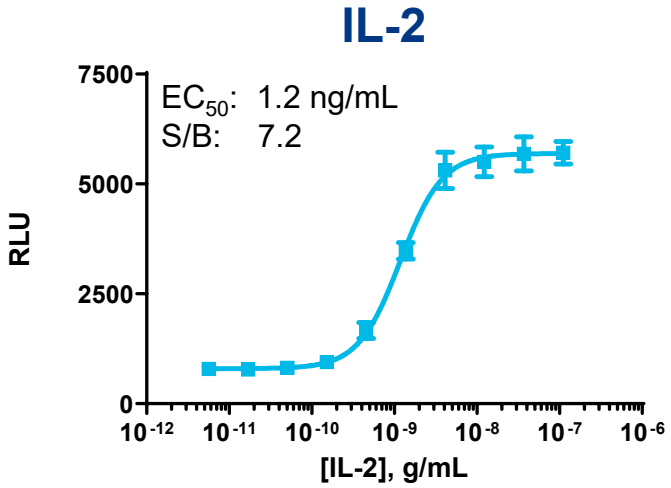
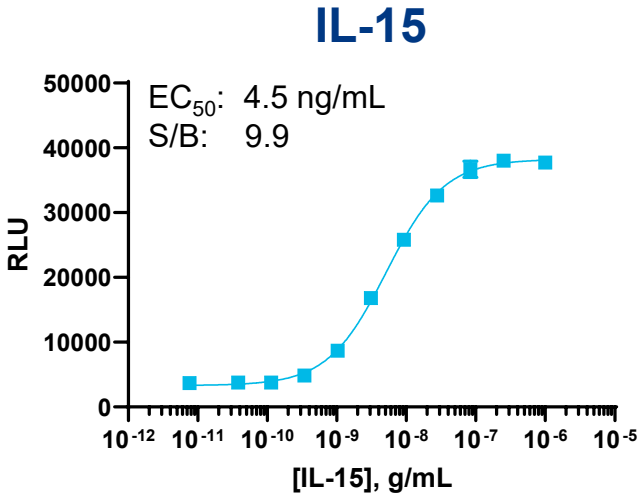
PathHunter® receptor dimerization assays quantify a discrete early step in receptor signaling (proximal)

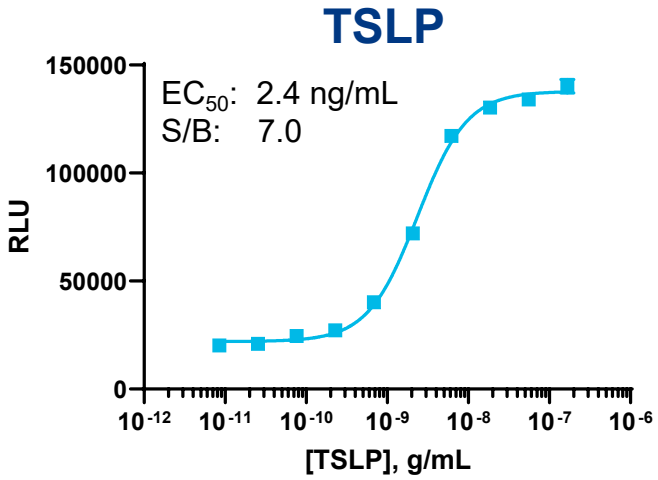
Example: IL-23 Receptor Dimerization

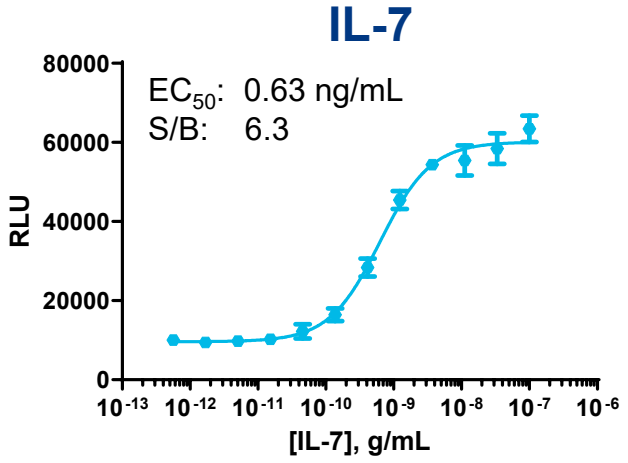












EC_{50} = Effective Concentration at 50% of ligand S/B = signal-to-background (assay window)

Signaling Reporter Assay Design

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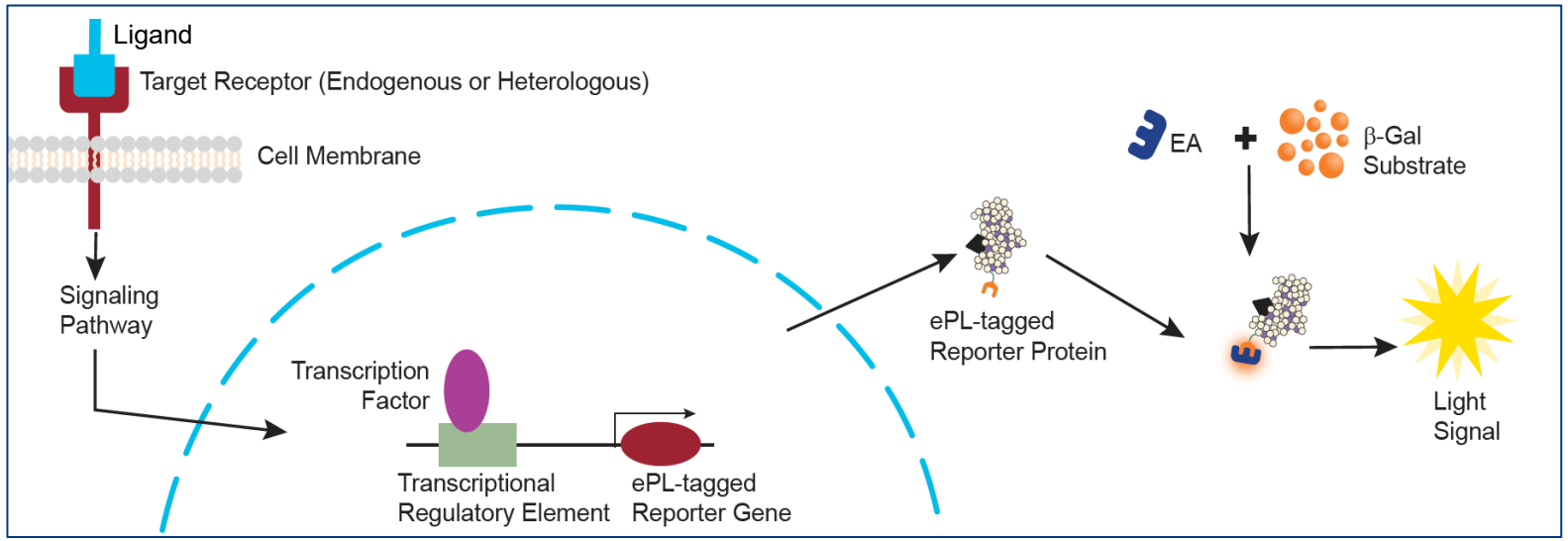
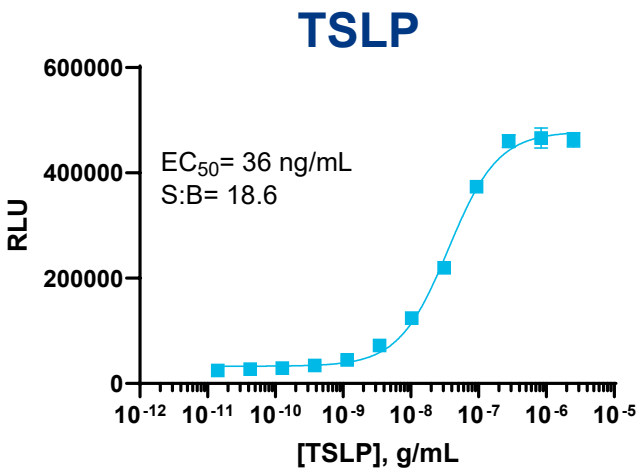
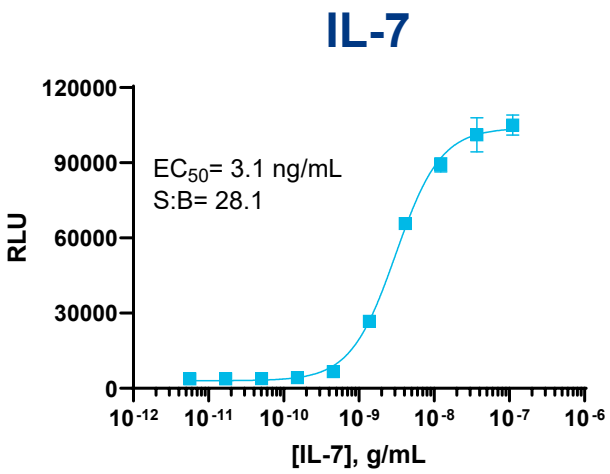
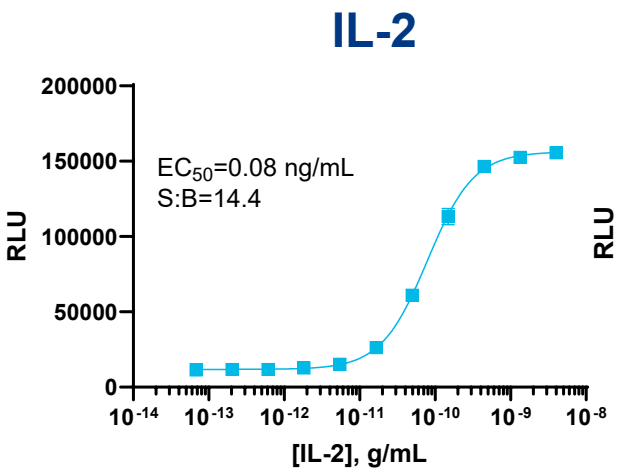
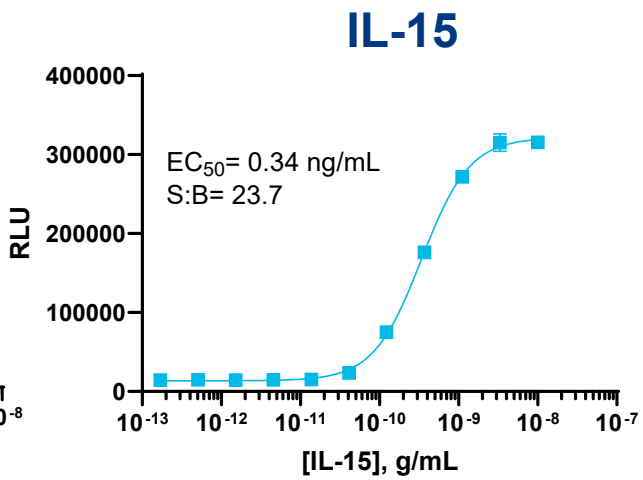
PathHunter® signaling reporter assays quantify a distal receptor signaling event (transcriptional activation)











Comparison of Receptor Dimerization vs. Signaling Reporter Assays

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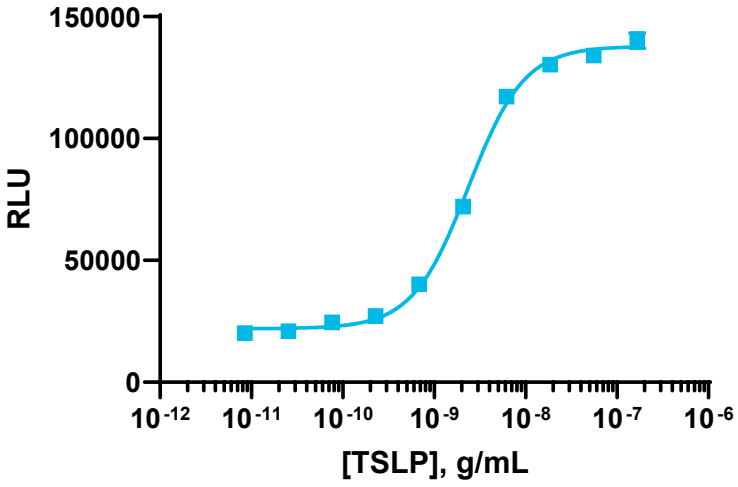
Assay window and sensitivity to TSLP (ligand) in different assay formats

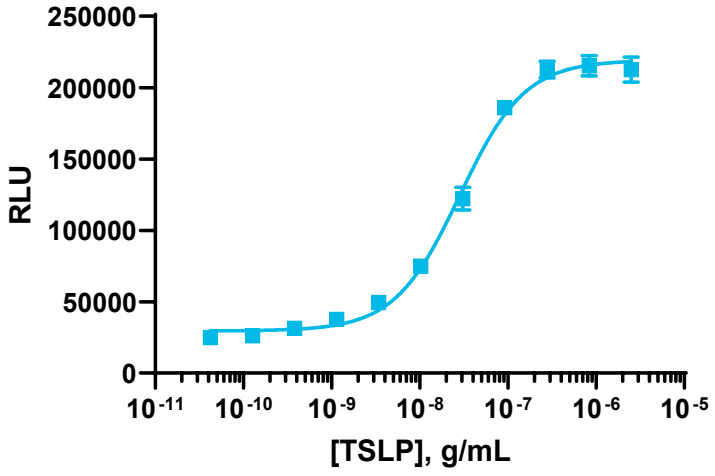
The assay window in response to TSLP is slightly larger in the reporter assay.

The EC_{50} is 10-fold lower in the dimerization assay.

PathHunter® TSLP Dimerization Cell Line (DLD1)



PathHunter TSLP Signaling Reporter Cell Line (REH)



Assay Format →	Dimerization Cell Line	Signaling Reporter Cell Line
Cell Background	DLD1	REH
Receptor Subunits: • IL7RA • CRLF2	• Engineered (Truncated) • Engineered (Truncated)	• Endogenous • Engineered (Full-length)
Assay Window (S/B)	7.0	8.5
EC_{50} (ng/mL)	2.4	28.4
Incubation Time (hours)	16	16

Comparison of Receptor Dimerization vs. Signaling Reporter Assays

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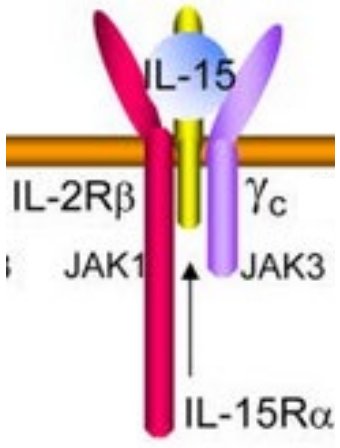
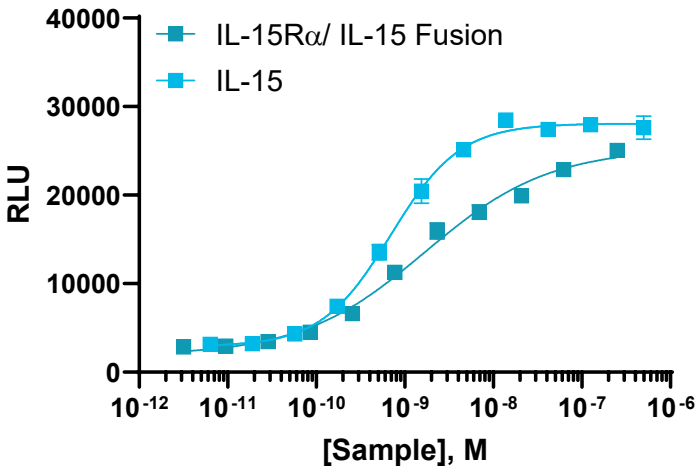
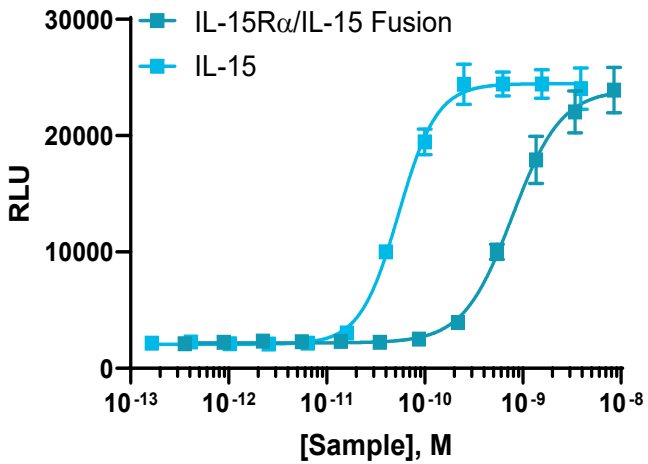
Assay window and sensitivity to IL-15 (ligand) in different assay formats



PathHunter® IL-15 Bioassay (Dimerization)



PathHunter IL-15 Bioassay (Reporter)



The assay window is greater in the reporter assay and exhibits greater potency (lower EC_{50}).

Assay Format →	Dimerization Bioassay	Reporter Bioassay
Cell Background	U2OS	REH
Receptor Subunits: • IL2RB • IL2RG	• Engineered (Truncated) • Engineered (Truncated)	Engineered (Full-length) Engineered (Full-length)
Assay Window (S/B): IL-15/IL-15Ra-IL-15 fusion protein	8.8 / 8.7	14.7 / 14.7
EC_{50} (nM): IL-15/IL-15Ra-IL-15 fusion protein	0.69 / 1.7	0.054 / 0.78
Incubation Time (hours)	18-20	16-18

Receptor Dimerization vs. Signaling Reporter Assays

Evaluating Ligand Specificity

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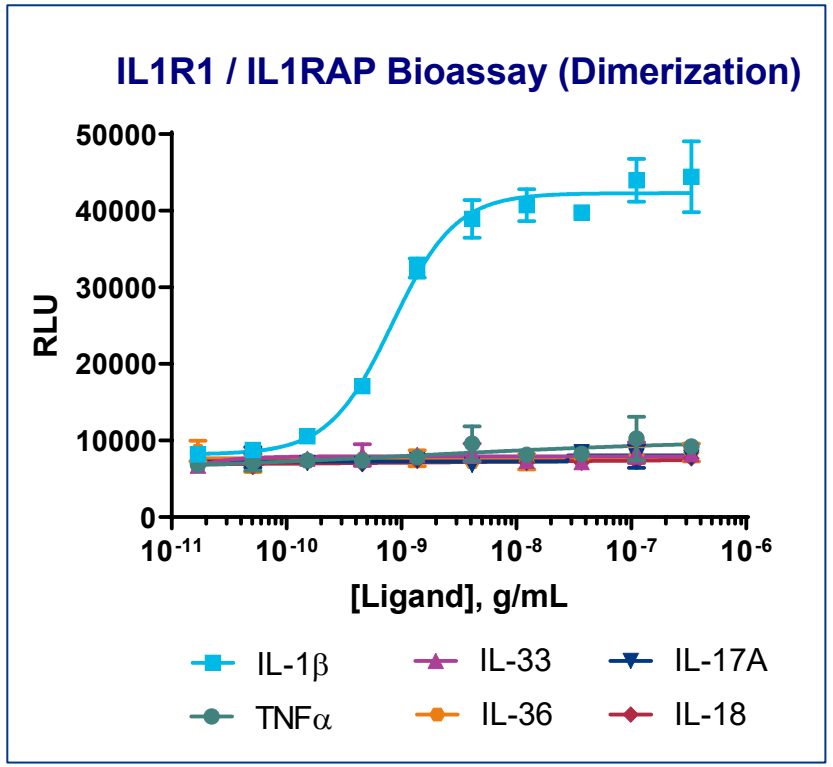
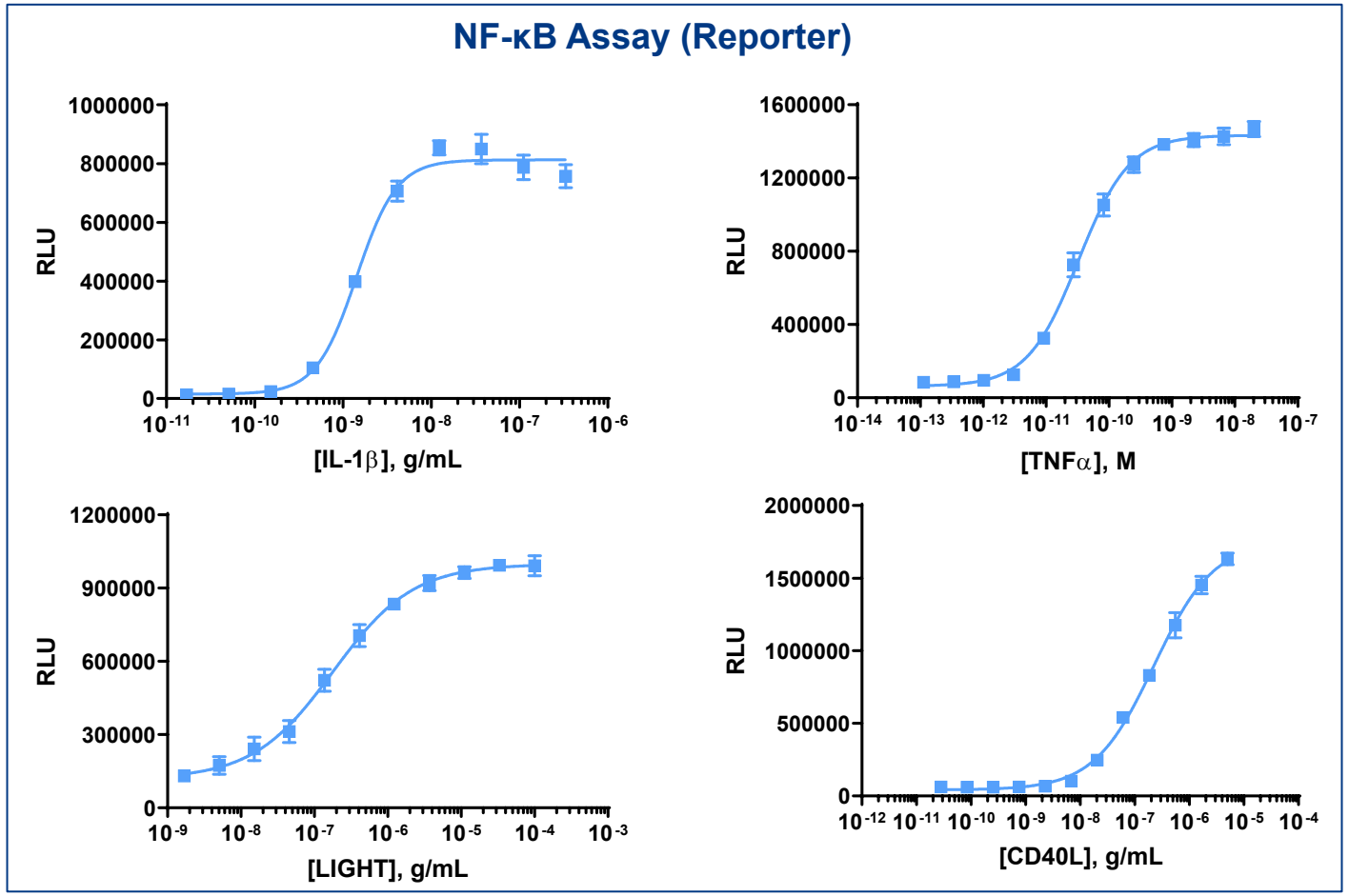
Dimerization assay responses are specific, while reporter assays can respond to multiple cytokine signaling pathways





Because of the receptor combination used, the assay response is cytokine-specific

The same reporter gene can be activated by many different cytokines/ligands

Validated Therapeutic Targets for Crohn's Disease, Plaque Psoriasis, & Psoriatic Arthritis

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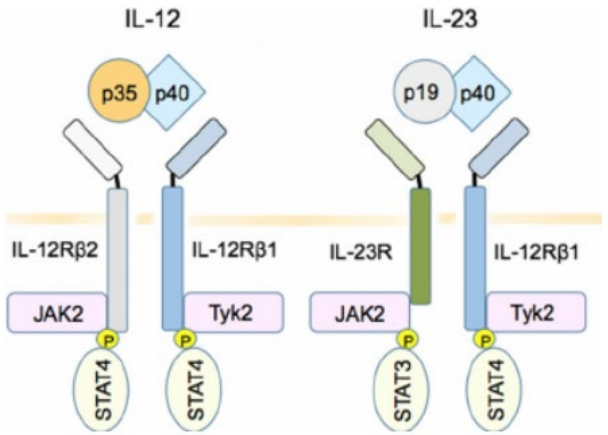
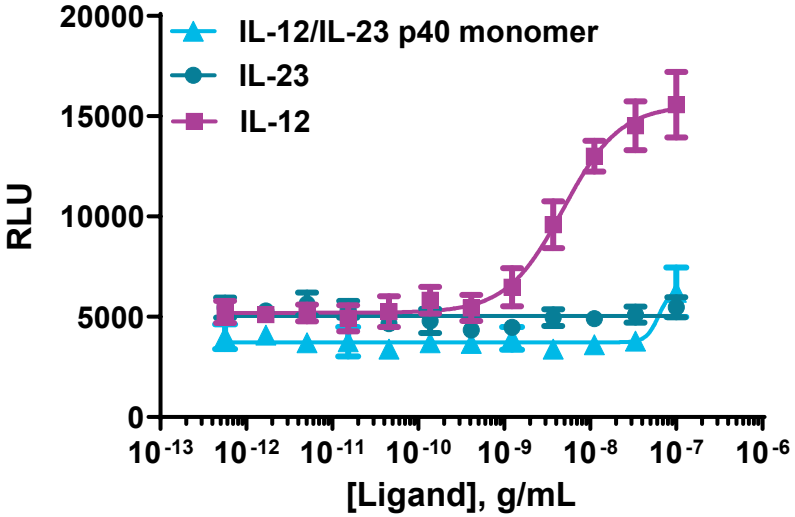
IL-12 and IL-23 dimerization assay specificity

High specificity observed for cognate ligand in each assay compared to little or no specificity observed with p40 monomer (a subunit shared by both ligands).



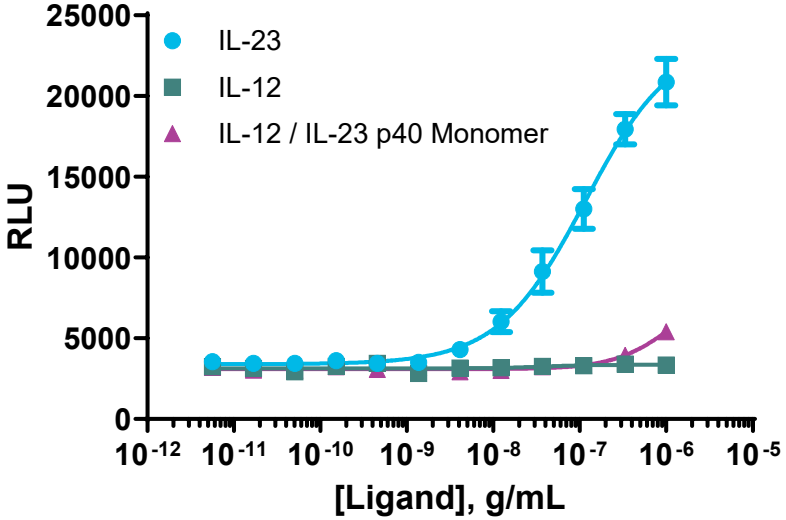
IL-12 Receptor

PathHunter® IL-12RB1/IL-12RB2 Cell Line



IL-23 Receptor

PathHunter IL-23/IL-12RB1 Cell Line



Therapeutic Targeting p40 Subunit of IL-23 & IL-12 Is Active in Both Assays

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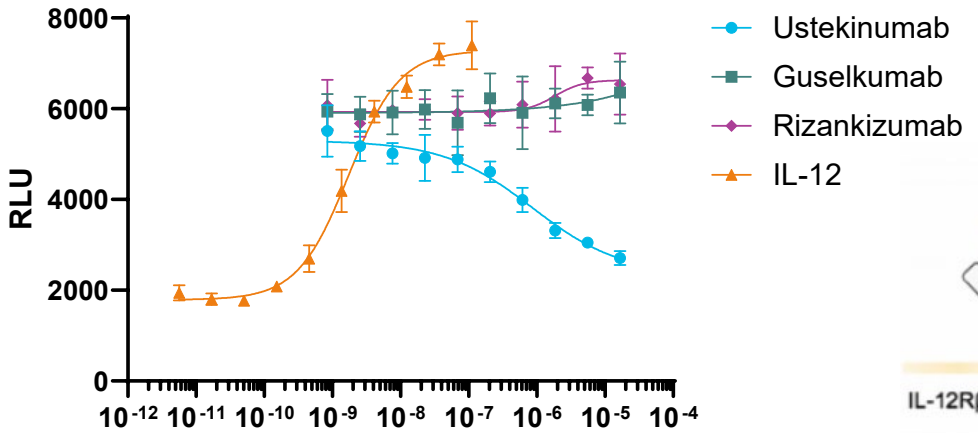
Similar potency for key therapeutic antibodies targeting p19 subunit of IL-23 in the IL-23 receptor assay, but only Ustekinumab is active in IL-12 receptor assay

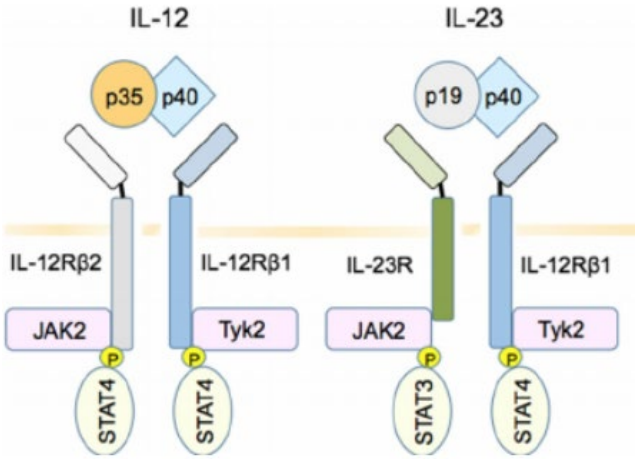
IL-12 Receptor

PathHunter® IL-12RB1/IL-12RB2 Cell Line



[Antibody], g/mL + 16 ng/mL IL-12, or [IL-12], g/mL

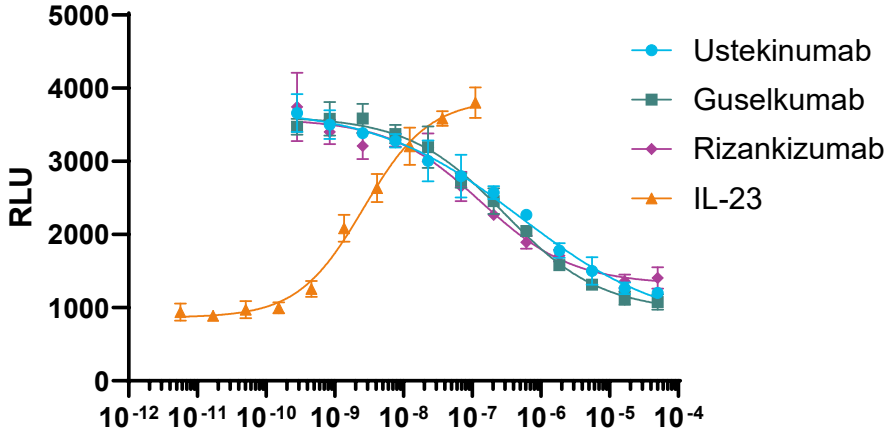
Antibody/Cytokine	Assay Window (S/B)	EC_{50} (ng/mL)
Ustekinumab	2.0	857
Guselkumab	-	-
Rizankizumab	-	-
IL-12	3.8	1.7



Ustekinumab (Stelara®) = anti-p40
Guselkumab (Tremfya®) = anti-p19
Rizankizumab (Skyrizi®) = anti-p19

IL-23 Receptor

PathHunter IL-23/IL-12RB1 Cell Line



[Antibody], g/mL +16 ng/mL IL-23 or [IL-23], g/mL

Antibody/Cytokine	Assay Window (S/B)	EC_{50} (ng/mL)
Ustekinumab	3.0	511
Guselkumab	3.3	298
Rizankizumab	2.8	133
IL-23	4.0	2.6

Signaling Reporter Assay Measure IL-4 Activation of Both Type I & Type II IL-4 Receptor Complexes

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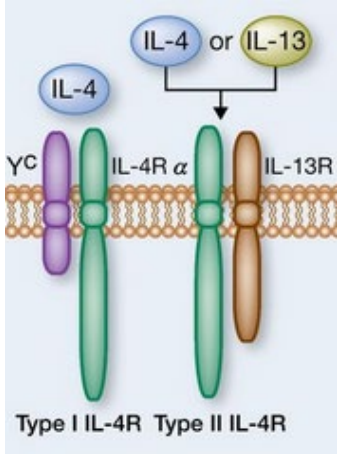
IL-4 receptor (IL-4R) complexes signaling via STAT6

- Type I IL-4R
  - IL-4R + IL-2RG (γC)
  - Expressed in immune cells and binds only IL-4
- Type II IL-4R
  - IL-4R + IL-13Rα
  - Expressed in epithelial cells and binds both IL-4 and IL-13

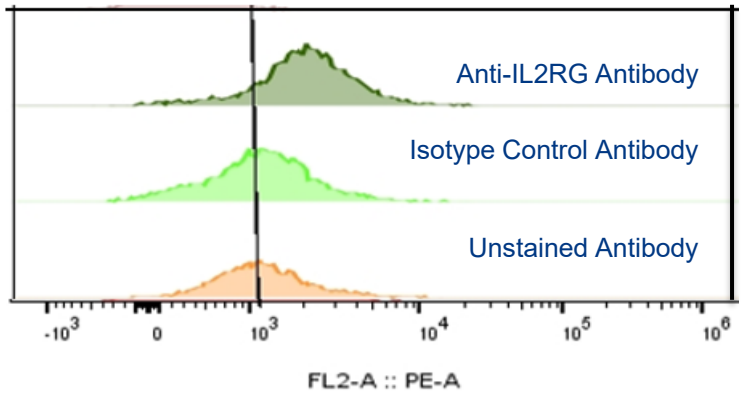
The PathHunter® IL-4/IL-13 signaling reporter assay was used to quantify IL-4 and IL-13 signaling

- Flow cytometry data indicates IL2RG (γC) receptor is also expressed in the cell line

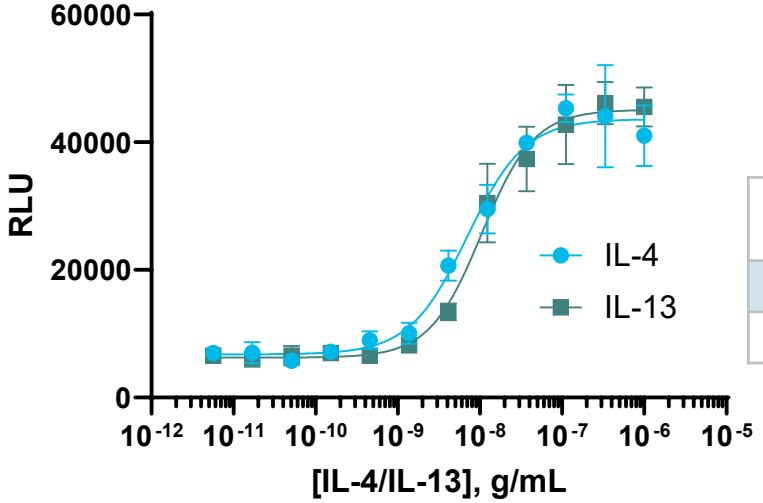
IL-4 results measure combined signaling from both Type I and Type II receptor complexes



Flow Cytometry Analysis



PathHunter IL-4/IL-13 Signaling Reporter Cell Line



Cytokine	Assay Window (S/B)	EC_{50} (ng/mL)
IL-4	6.3	10.0
IL-13	7.7	61.4

IL-4R Dimerization Assays Are Specific for Either Type I or Type II Receptor Complexes

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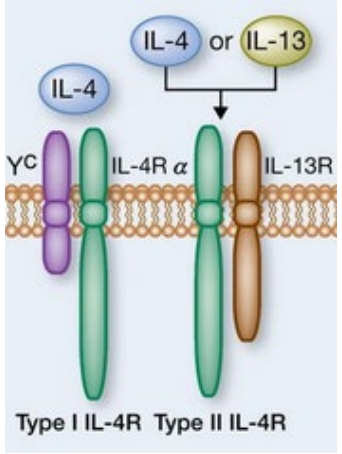
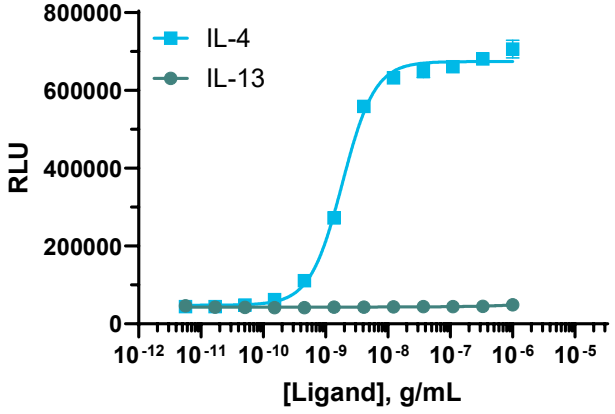
IL-4 Receptor Dimerization Cell Lines can be used to measure signaling specific for each IL-4 receptor complex

- IL-4 variants that induce exclusively Type I– or Type II–dependent responses could preserve the benefits of IL-4 immunotherapy, but with reduced side effects.



Type I IL-4R: Inflammation

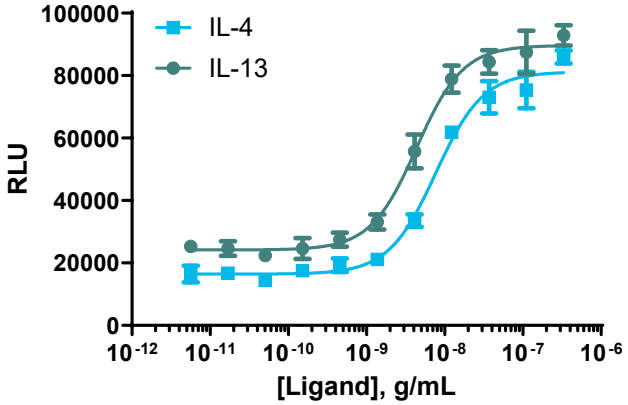
PathHunter® IL-4R/IL-2RG Dimerization Cell Line



Cytokine	S/B	EC_{50} (ng/mL)
IL-4	16	1.9
IL-13	-	-

Type II IL-4R: Oncology & Inflammation

PathHunter IL-4R/IL-13R Dimerization Cell Line



Cytokine	S/B	EC_{50} (ng/mL)
IL-4	5.2	7.7
IL-13	3.8	4.4

Dupilumab, an Approved Therapeutic for Eczema & Asthma, Blocks IL-4 & IL-13 Signaling through Both Type I & II IL-4 Receptors

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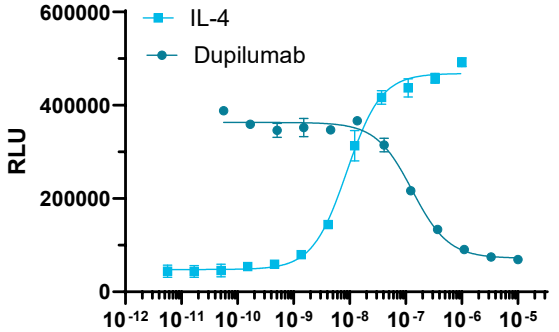
Receptor Dimer

Type I Receptor (+ IL-4)

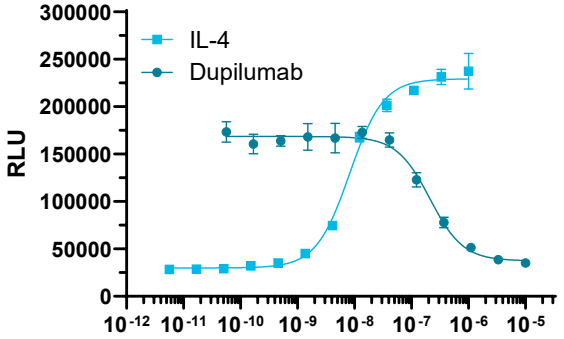
PathHunter IL4R / IL2RG Dimerization Cell Line



[Dupilumab], g/mL +13 ng/mL IL-4 or [IL-4], g/mL

Type II Receptor (+ IL-4)

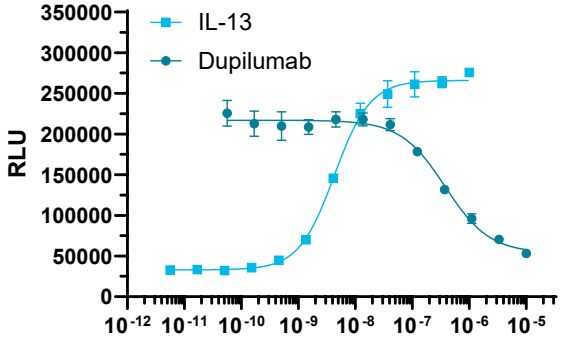
PathHunter IL4R / IL13R Dimerization Cell Line



[Dupilumab], g/mL + 12ng/mL IL-4 or [IL-4], g/mL

Type II Receptor (+ IL-13)

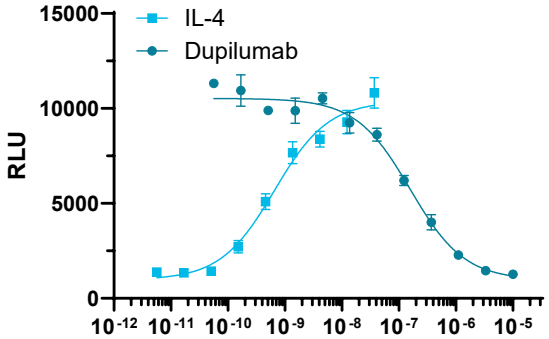
PathHunter IL4R / IL13R Dimerization Cell Line



[Dupilumab], g/mL +15 ng/mL IL-13 or [IL-13], g/mL

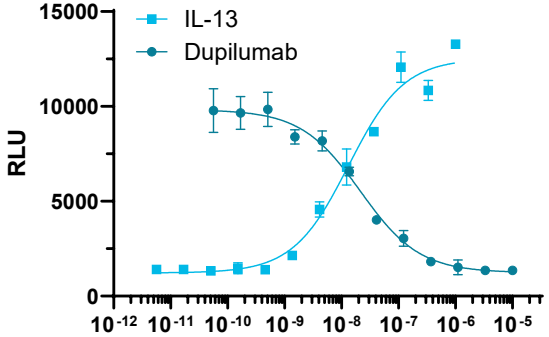
Reporter

PathHunter IL-4 / IL-13 Signaling Reporter Cell Line



[Dupilumab], g/mL + 33 ng/mL IL-4 or [IL-4], g/mL

PathHunter IL-4 / IL-13 Signaling Reporter Cell Line



[Dupilumab], g/mL + 47ng/mL IL-13 or [IL-13], g/mL

	IL-4		Dupilumab+IL-4		IL-13		Dupilumab+IL-13	
Cell Line	S/B	EC_{50} (ng/mL)	S/B	IC_{50} (ng/mL)	S/B	EC_{50} (ng/mL)	S/B	IC_{50} (ng/mL)
IL4R-IL13R Dimerization	8.3	8.4	4.9	210	8.4	4.8	4.2	378
IL4R-IL2RG Dimerization	11.2	8.9	5.6	130	NA	NA	NA	NA
IL-4/IL-13 Signaling Reporter	11.4	0.65	8.9	157.5	9.5	13.2	7.7	21.4

Secukinumab Inhibits IL-17A-mediated Dimerization

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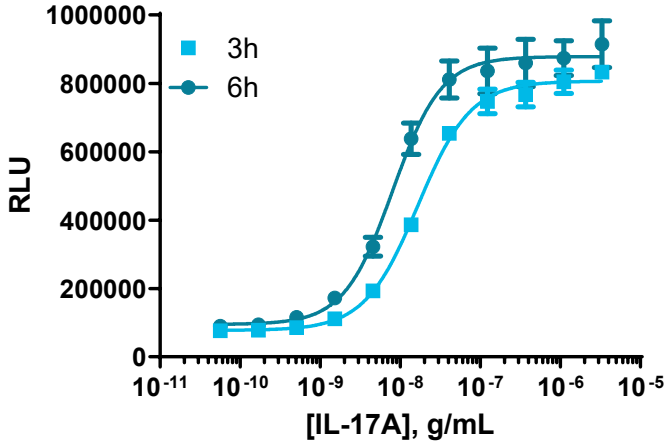
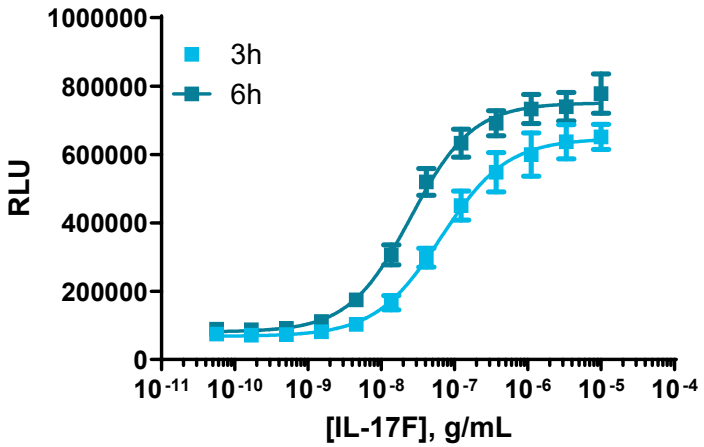
Cosentyx® (Secukinumab), which binds to IL-17A, is the first anti-IL-17A therapeutic approved for treatment of plaque psoriasis and psoriatic arthritis

PathHunter® IL-17RA/IL-17RC Dimerization Cell Line Time Course & Inhibition Experiments

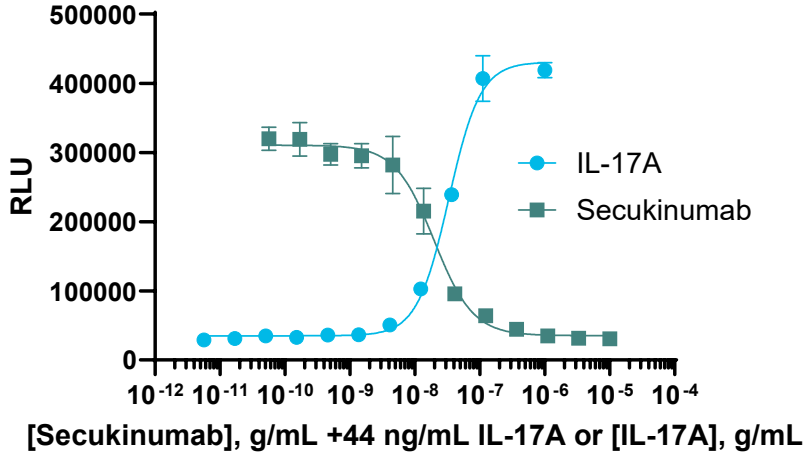
Time Course with Ligand (IL-17A and IL-17F)





Cytokine (time point)	Assay Window (S/B)	EC_{50} (ng/mL)
IL-17A, 3h	11.6	16.6
IL-17A, 6h	11.6	8.1
IL-17F, 3h	8.8	65.4
IL-17F, 6h	8.8	25.2

Inhibition with Secukinumab



Antibody/Cytokine	Assay Window (S/B)	EC_{50} (ng/mL)
IL-17A	14.5	33.3
Secukinumab	10.4	19.8

Receptor Dimerization Assays Maintain Robust Activity in the Presence of Normal Human Serum

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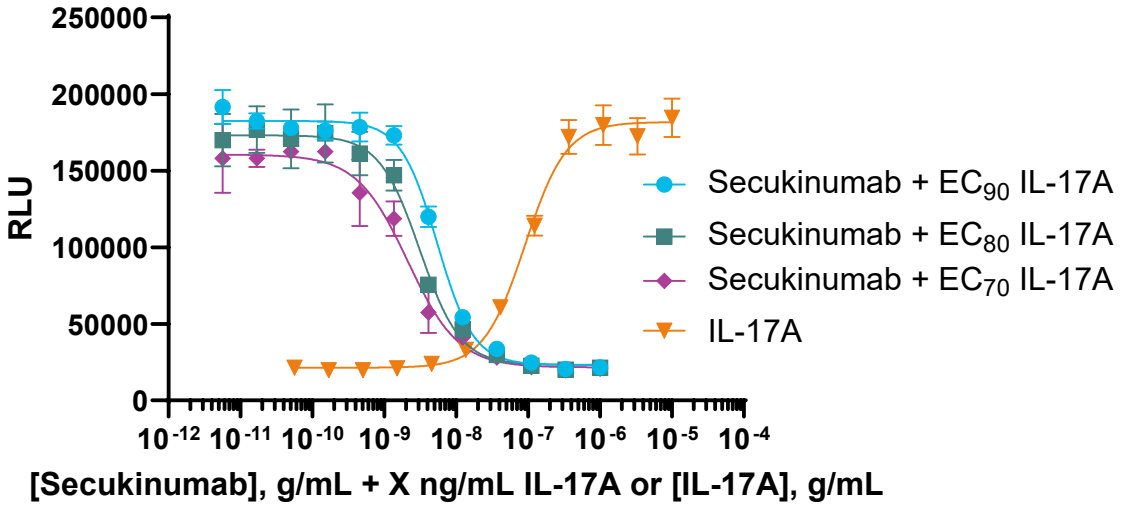
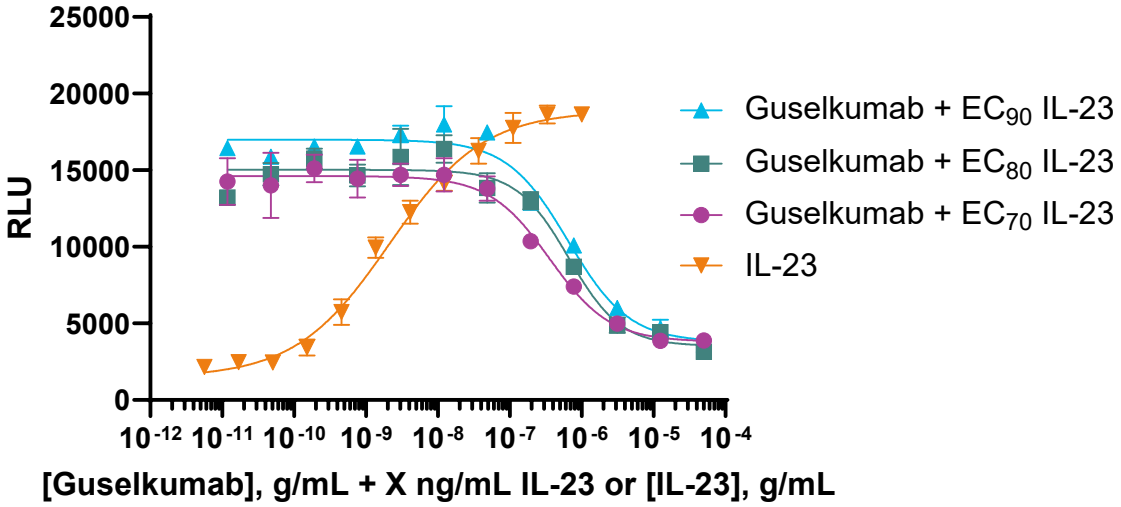
Dimerization assays produce robust agonist and antagonist responses in the presence of 10% normal human serum (NHS), which is required for quantifying the presence of anti-drug neutralizing antibodies (NAb) in human serum samples.

Dimerization Assay Experiments with Samples Prepared in 10% Pooled NHS

PathHunter® IL-17RA/IL-17RC Dimerization Cell Line



PathHunter IL-23/IL-12RB1 Dimerization Cell Line



Quantitation of NAb to Secukinumab

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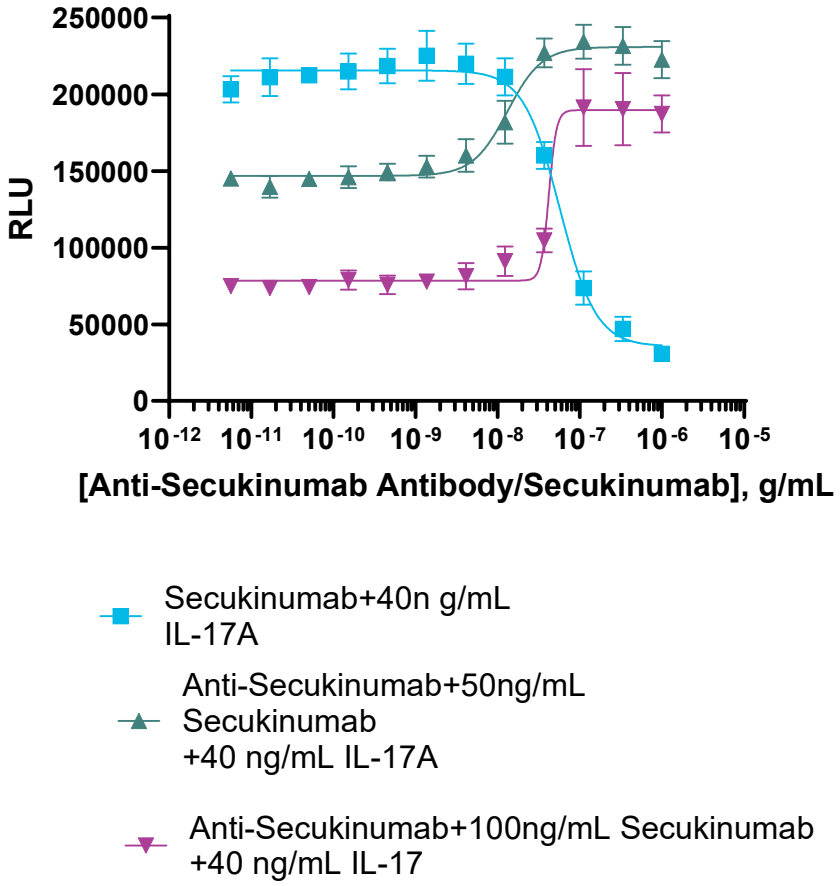
Dimerization Assay Experiment with Samples Prepared in 10% Pooled NHS

PathHunter® IL-17RA/IL-17RC Dimerization Cell Line



Antibodies/Cytokine	Assay Window (S/B)	EC / IC / NC_{50} (ng/mL)
IL-17A	9.5	8.4
Secukinumab +40ng/mL IL-17A	6.6	57
Anti-Secukinumab+50ng/mL Secukinumab+40ng/mL IL-17A	1.5	13
Anti-Secukinumab+100ng/mL Secukinumab+40ng/mL IL-17A	2.5	42

FDA guidelines specify a minimum sensitivity of 1 µg/mL for detection of NAbs in human serum

An anti-Secukinumab antibody exhibits potent neutralizing activity of Secukinamb IL-17A inhibition and demonstrates application of the assay for Nab antibody screening of patient serum.

The IL-1β Reporter Assay Is More Sensitive to the Presence of NHS Format than the Dimerization Assay

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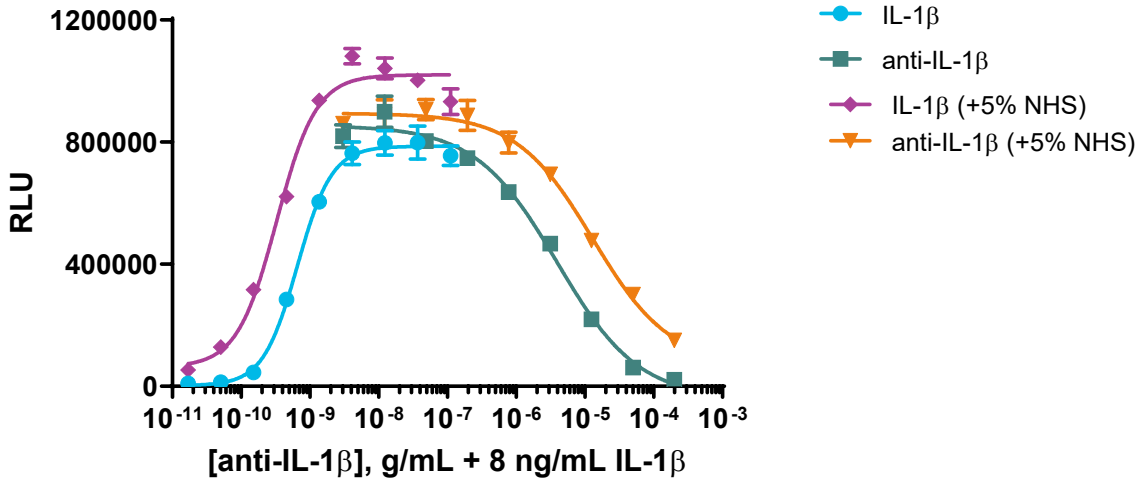
Attenuated assay windows and increased IC_{50} are observed with the IL-1β reporter assay format in the presence of NHS while dimerization assay results are similar in the presence or absence of NHS

IL-1β Reporter Assay

U2OS NF-κB-Reporter Cell Line
CP5, 5K/well; 6hr ligand @ 37°C

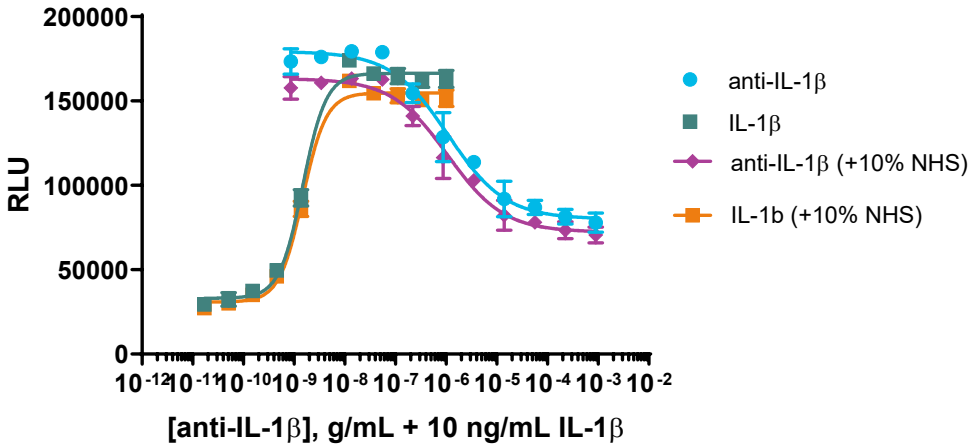


Sample	PBS		+ 5% NHS	
	S/B	EC/ IC_{50}, ng/mL	S/B	EC/ IC_{50}, ng/mL
IL-1β (agonist mode)	**50.1**	0.66	**19.5**	0.33
Anti-IL-1β + 8 ng/mL IL-1β	**40.3**	4,445	**5.7**	13,020

Significant loss of assay window and increased IC_{50} with anti-IL-1β drug in 5% NHS

IL-1β Receptor Dimerization Assay

U2OS ILR1A / IL1RAP Bioassay
Part #: 93-1032Y3; Lot #: 17C2411
CP5; O/N recovery; 30' premix @ R/T; 16h ligand @ 37°C



Sample	PBS		+ 10% NHS	
	S/B	EC/ IC_{50}, ng/mL	S/B	EC/ IC_{50}, ng/mL
IL-1β (agonist mode)	**6.0**	1.49	**5.5**	1.49
Anti-IL-1β + 10 ng/mL IL-1β	**2.2**	1,104	**2.2**	1,082

No change in assay window or IC_{50} with anti-IL-1β drug in 10% NHS

Development of Cytokine Assays for Relative Potency, Characterization & Lot Release Applications

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Reflective of MOA of the drug/molecule

Stability-indicating

Relative potency range (50%-150%)

Repeatable, robust, precise, and accurate

Reproducible

- Intra-lot (plate-to-plate)
- Inter-lot (lot-to-lot)
- Analyst-to-analyst

Long-term support – Dedicated well characterized cell banks

Supply agreements

Traceability and quality (audit-ready)

ICH-based Assay Qualification

MOA = mechanism of action; ICH = International Council for Harmonization

Relative Potency Analysis for the Tocilizumab (IL-6) Bioassay

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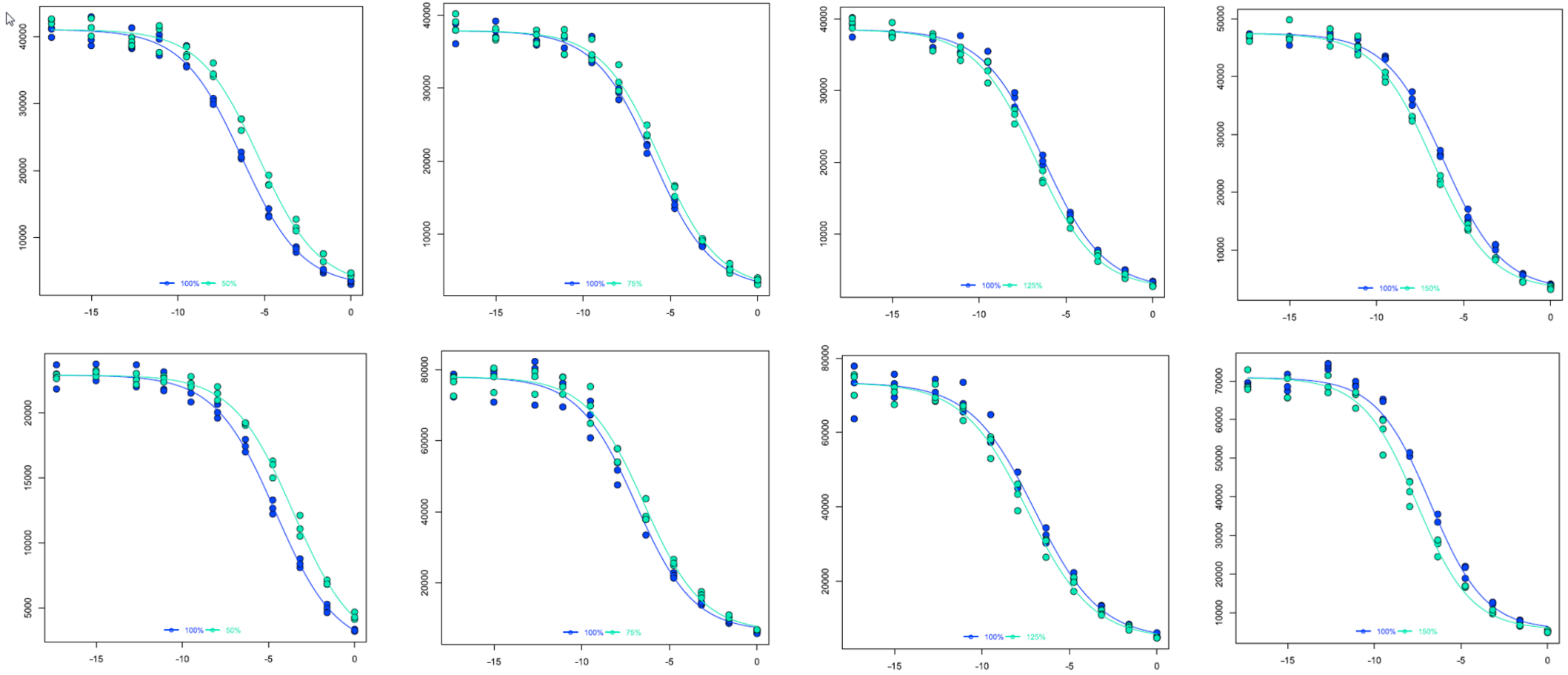
Relative potency analysis for 4 nominal concentrations of tocilizumab

ACTEMRA® tocilizumab

50% **75%** **125%** **150%**

Analyst 1

Analyst 2



Reference

Sample

RP values calculated in PLA 3.0. All registered trademarks are the property of their respective owners.

Tocilizumab (IL-6) Bioassay Demonstrates Good Accuracy, Intermediate Precision, & Dilutional Linearity

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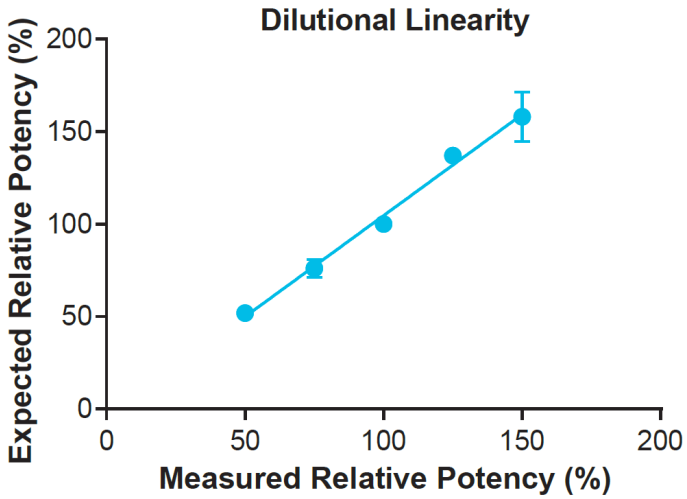
ACTEMRA® tocilizumab

Qualification of Actemra® using the Tocilizumab Bioassay

Experiment #	Analyst #	Expected RP (%)	Observed RP (%)	Average RP (%)	% RSD	% Recovery
1	1	150	155.5	158.1	8.44	105.4
2	1		163.2			
3	1		141.1			
4	2		172.7			
1	1	125	138.6	137.1	1.66	109.7
2	1		137.4			
3	1		138.6			
4	2		133.8			
1	1	75	74.1	76.1	6.34	101.5
2	1		83			
3	1		75.4			
4	2		71.9			
1	1	50	54.8	52	4.96	103.9
2	1		50.6			
3	1		53.3			
4	2		49.1			



Accuracy: 105.1%

Intermediate Precision: ≤ 8.4%

Dilutional Linearity: R^2= 0.9927

Eurofins DiscoverX Cytokine Assay Portfolio

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Functional, cell-based assays available for most targets in cell line or assay ready formats (eXpress™ & bioassay kits)

IL-1-like

IL-1
IL-18
IL-33

IL-28-like

IL-28
IL-29

IL-17-like

IL-17
IL-25

IL-4-like

IL-3
IL-4
IL-5
IL-13
CSF2

Non-classified

IL-8
IL-34
CSF1

IL-6/12-like

IL-6
IL-11
IL-12
IL-23
IL-27
IL-31
CSF3

γ-chain utilizing

IL-2Rαβγ
IL-2Rβγ
IL-4
IL-7
IL-9
IL-15
IL-21
TSLP

IL-10-like

IL-10
IL-19
IL-20
IL-22
IL-24
IL-26

Other Cytokines

TNFα, IFNα, IFNβ, IFNγ, CXCRs, LIF, OSM, G-CSF, GM-CSF, RANK

Chemokines

CCR1	CCR10
CCR5	CCR6
CCR4	CCR9
CXCR3	CXCR4
CXCR2	CXCR7
CCR2	CCR3
CCR7	CCR8
CCRL1	CCRL2
CXCR5	CXCR6
CX3CR1	CXCR1

Summary

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PathHunter® Cytokine Receptor Assays

- MOA-based dimerization, signaling reporter, and other assay types covering over 80% of targets
- Available in stable cell line or ready-to-use eXpress™ and bioassay kit formats
- Used from discovery through development and into commercial release and stability QC lot testing
- Bioassays are target-based or qualified with a reference ligand or therapeutic

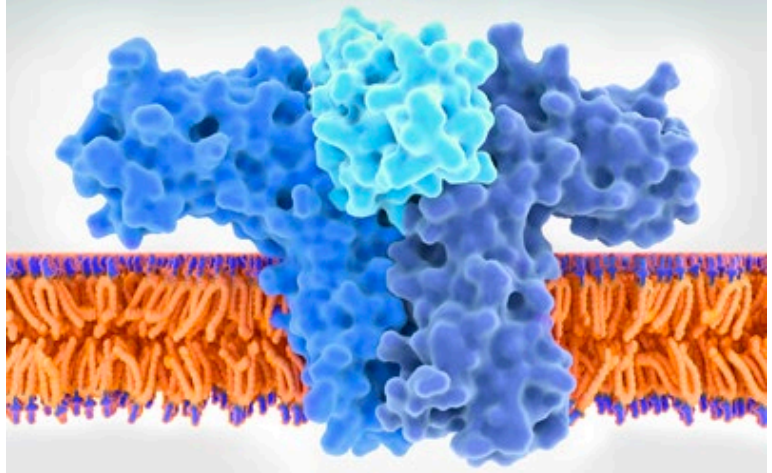
PathHunter Dimerization Assays

- Robust, sensitive, and highly specific assays
- Minimally impacted by the presence of human serum
- Suitable for both relative potency and NAb detection

PathHunter Reporter Assays

- Robust and sensitive but lack receptor specificity
- Provide large assay windows and good sensitivity to ligands
- Suitable for screening and relative potency assays

Cytokine Receptor Product Solutions



Learn more at discoverx.com/target-class/cytokine-receptor/