



PathHunter[®] Anakinra Bioassay

Qualified with Kineret[®]

93-1032Y3-00105 (2-Plate Kit)

93-1032Y3-00106 (10-Plate Kit)

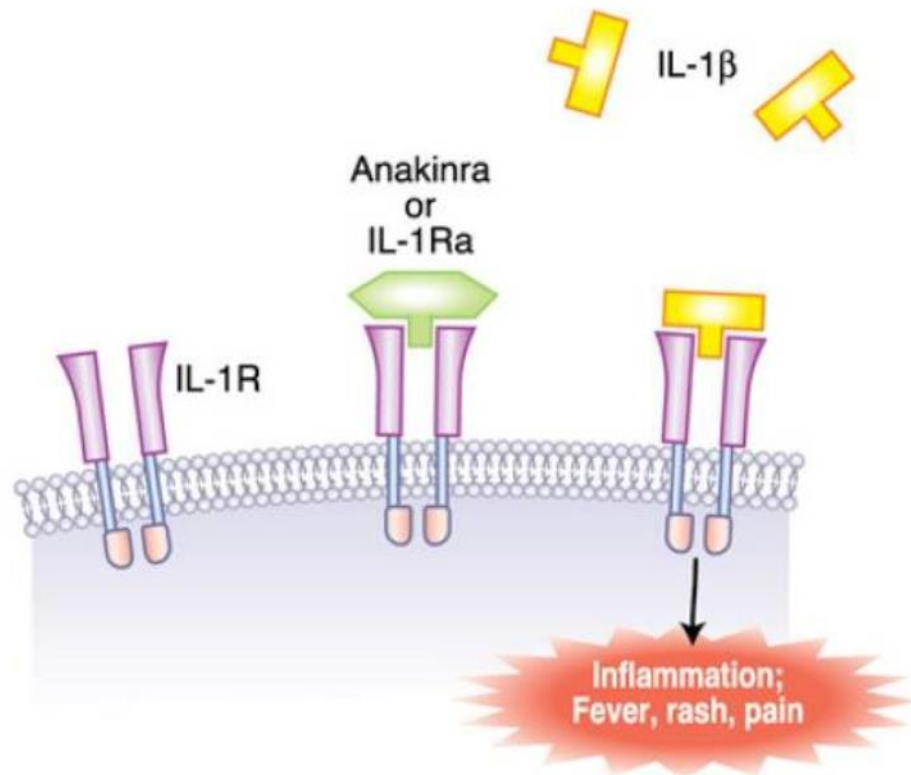
OUR EXPERTISE
IN YOUR HANDS.

DISCOVER
CONFIDENTLY.

Anakinra (Kineret[®])

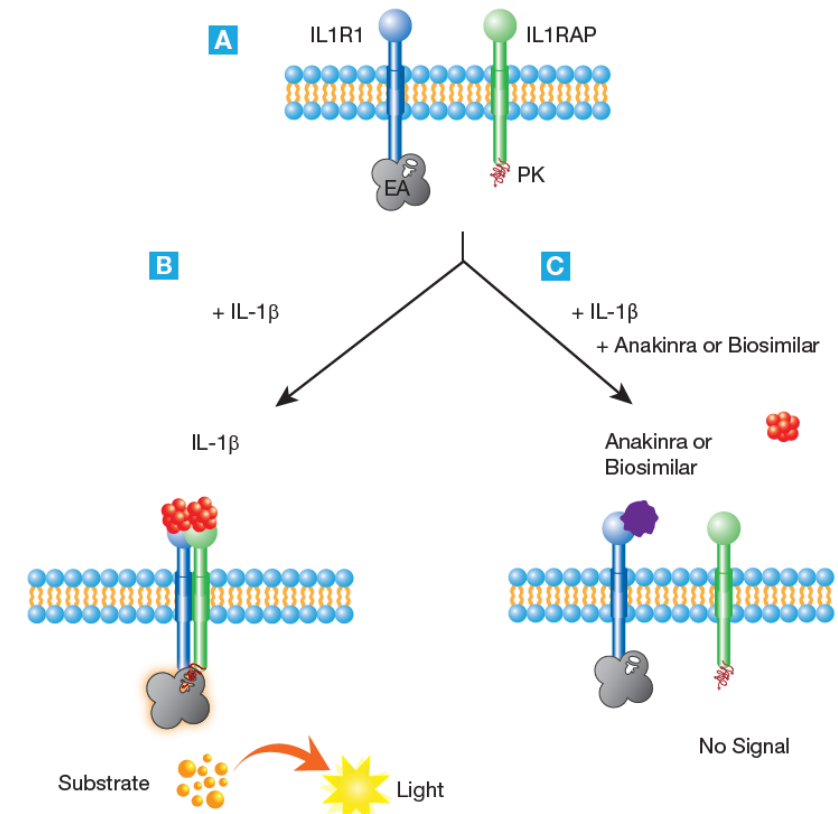
For Treatment of Rheumatoid Arthritis

Mechanism of Action



Source: Hoffman HM. (2009) Journal of Allergy and Clinical Immunology. Volume 124 (6), 1129-1138

Assay Principle



PathHunter[®] Anakinra Bioassay Kit

Kit Components

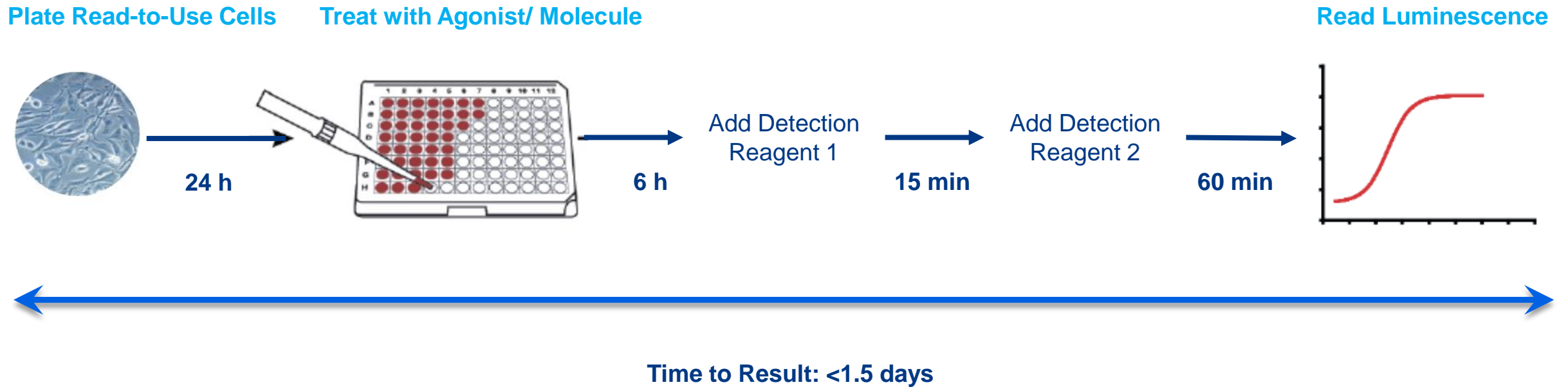
List of Components	93-1032Y3-00105	93-1032Y3-00106
PathHunter U2OS IL1R1/IL1RAP Bioassay Cells	2 vials	10 vials
PathHunter Bioassay Detection Kit		
Detection Reagent 1 (mL)	2	10
Detection Reagent 2 (mL)	8	40
AssayComplete™ Cell Plating Reagent 5	1 X 100 mL	3 X 100 mL
Protein Dilution Buffer	1 X 50 mL	2 X 50 mL
Control Agonist (IL-1 β)	1 vial	1 vial
96-Well Clear-Bottom TC Treated, Sterile Plates w/Lid	2 plates	10 plates

Sample data

Anakinra Bioassay Qualification

Bioassay Workflow

Simple, Homogenous and Rapid Protocol



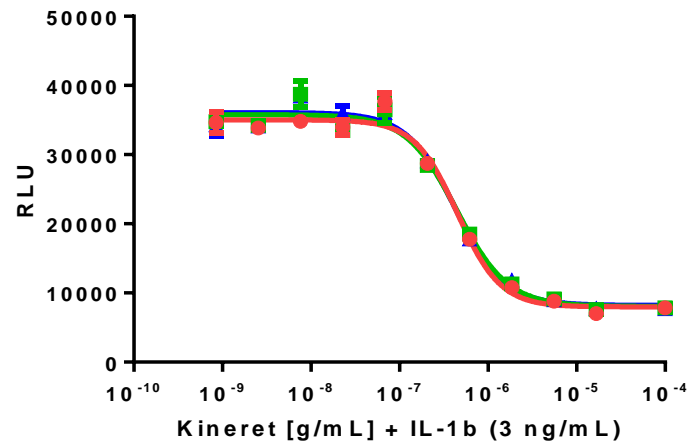
Assay Parameters Assessed

- % CV between 8 full plate DRCs
- Plate uniformity: EC_{80} and IC_{80} (of drug and stimulus) across entire plate
- Plate-to-Plate variability: 3 plates with full plate DRCs run on 3 days
- Slope consistency
- Relative potency across range of 50-150%
- Parallelism of relative potency data from two operators
 - Assay developer
 - Assay qualifier

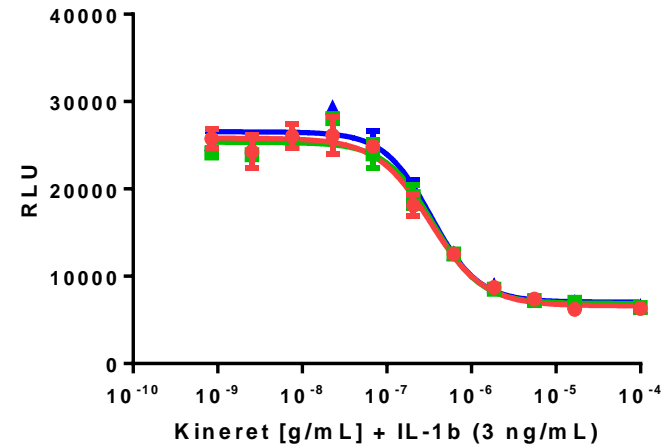
Anakinra Bioassay Qualification with Kineret®

Plate-to-Plate Variability: 3 Plates with Full-plate DRC - Same Day (D3)

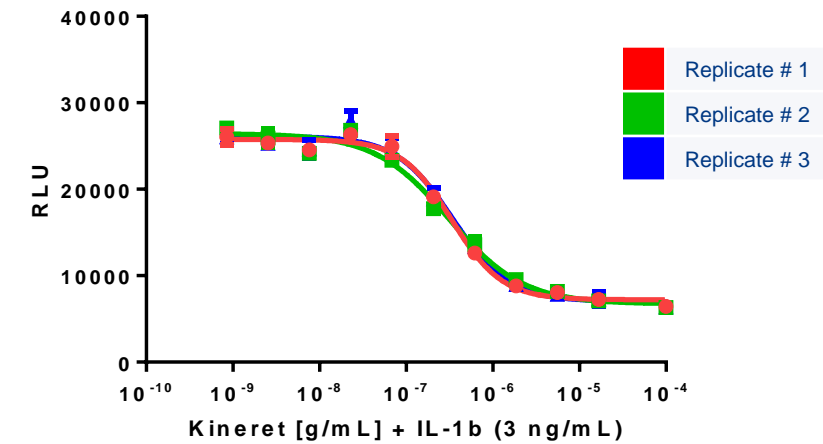
Plate# 1



Plate# 2



Plate# 3



Parameter	R1	R2	R3
S/B	4.4	4.5	4.5
Hill Slope	-1.789	-1.520	-1.578
IC ₅₀ (µg/mL)	45.13	44.39	43.06

Parameter	R1	R2	R3
S/B	4.7	4.4	4.6
Hill Slope	-1.369	-1.533	-1.533
IC ₅₀ (µg/mL)	32.50	34.89	35.53

Parameter	R1	R2	R3
S/B	4.2	4.4	4.5
Hill Slope	-1.487	-1.020	-1.340
IC ₅₀ (µg/mL)	33.36	31.15	34.40

Assay Robustness

Intra-Day Precision

Plate #	Exp #	R2	S:B	IC ₅₀ (µg/mL)	Mean IC ₅₀ (µg/mL)	SD (µg/mL)	%CV
1	R1	0.9872	4.5	0.45	0.37	0.06	15.1
	R2	0.9837	4.6	0.44			
	R3	0.9881	4.5	0.43			
2	R1	0.9837	4.7	0.33			
	R2	0.9782	4.4	0.35			
	R3	0.9779	4.6	0.34			
3	R1	0.9916	4.2	0.33			
	R2	0.9867	4.4	0.31			
	R3	0.9829	4.5	0.34			

Inter-Day Precision

Day	IC ₅₀ (µg/mL)	Mean IC ₅₀ (µg/mL)	SD (µg/mL)	%CV
1	0.44	0.37	0.06	17.04
2	0.34			
3	0.33			

Anakinra Bioassay Qualification with Kineret[®]

Parallelism and Potency Estimation (PLA)

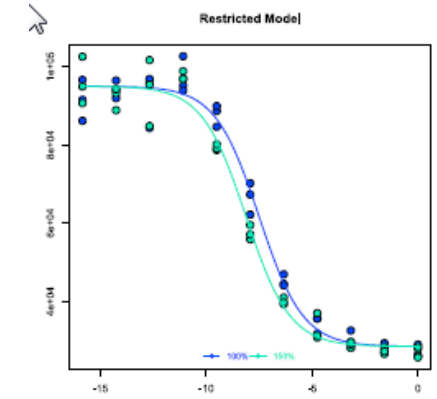
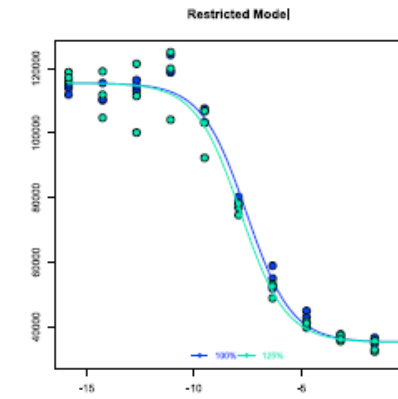
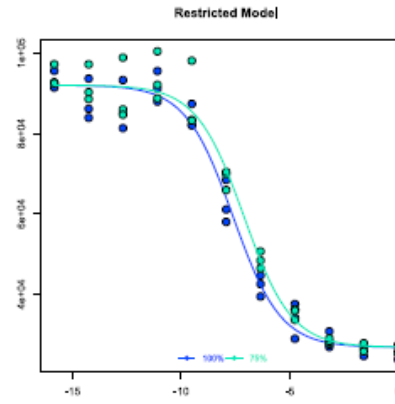
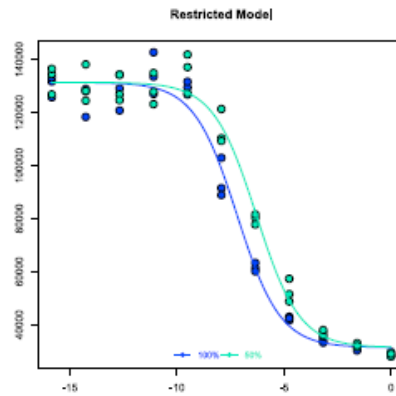
50%

75%

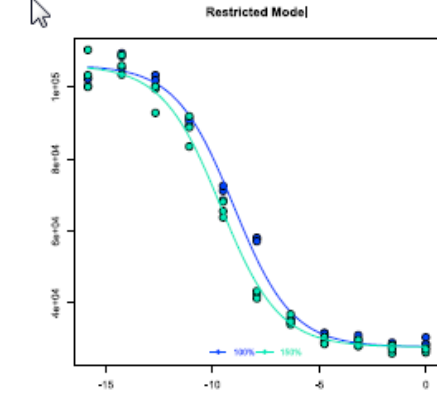
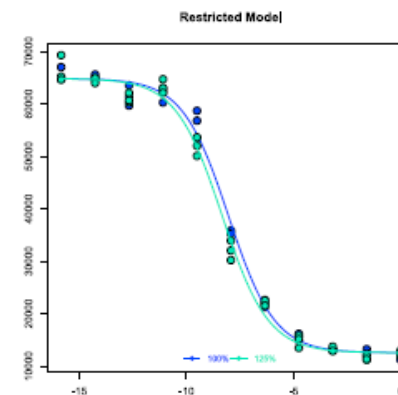
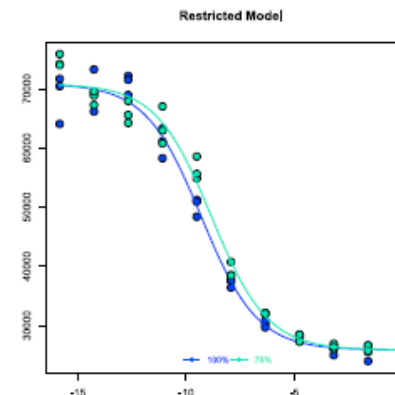
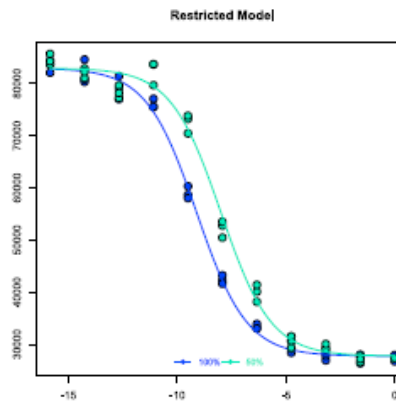
125%

150%

Analyst 1



Analyst 2

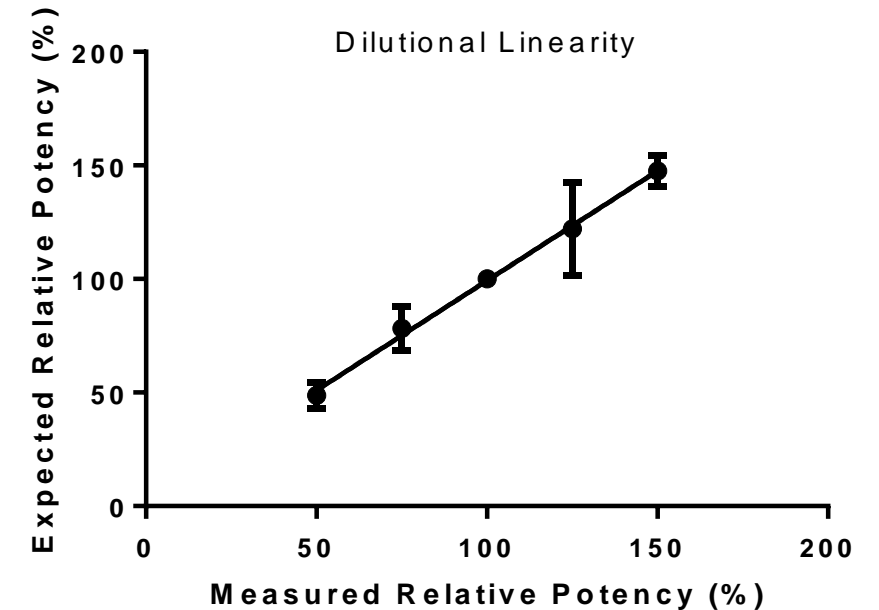


● Reference ● Sample

Anakinra Bioassay Qualification with Kineret®

Accuracy, Precision and Dilutional Linearity (2 Analysts): Summary

Exp #	Analyst #	Expected RP (%)	Observed RP (%)	Average RP (%)	% RSD	% Recovery
1	1	150	138.8	147.6	4.73	98.4
2	1		153.3			
3	1		145.2			
4	2		153.1			
1	1	125	147.9	121.6	16.83	97.3
2	1		97.9			
3	1		120.3			
4	2		122.3			
1	1	75	91.8	78.2	12.39	104.3
2	1		70.5			
3	1		78.3			
4	2		72.1			
1	1	50	53.6	48.7	11.74	97.4
2	1		53.3			
3	1		41.9			
4	2		46.1			



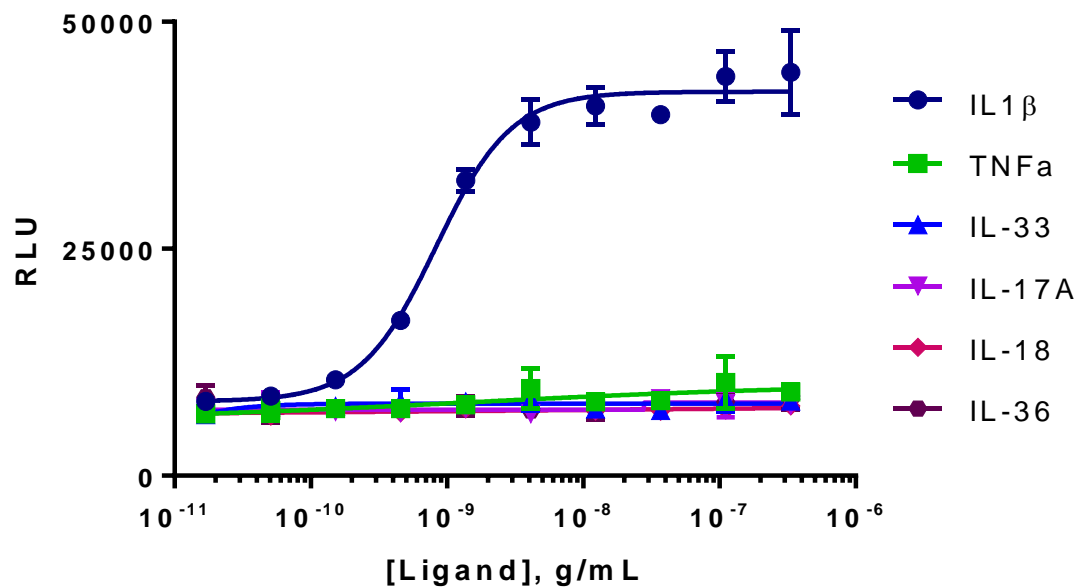
Accuracy: 99.4%

Precision: 11.4%

Linearity: 0.9667

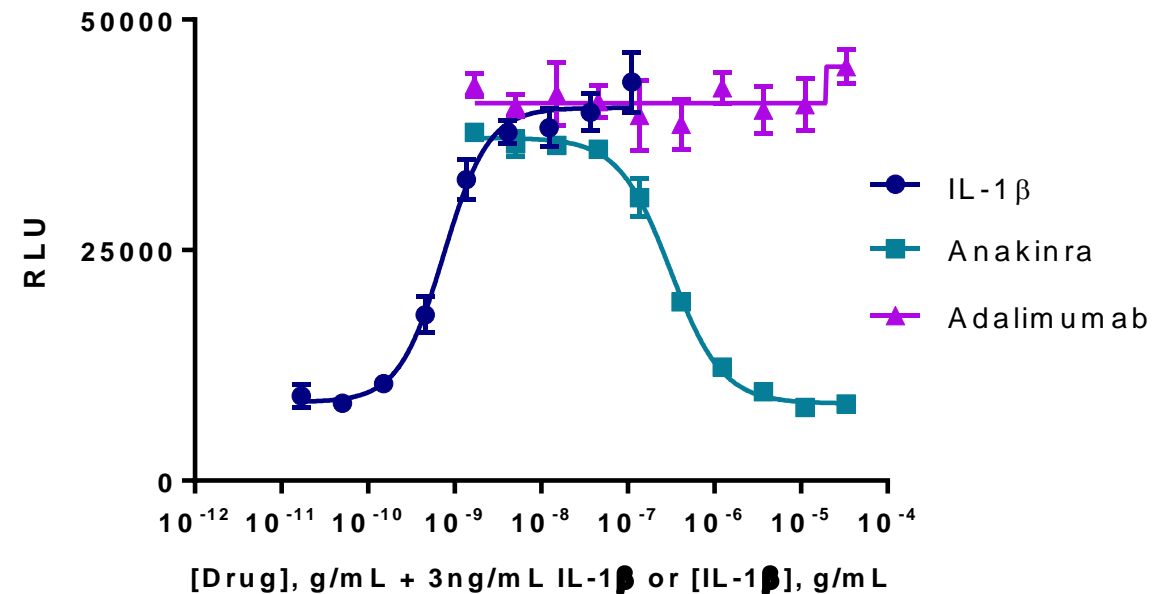
Anakinra Bioassay Specificity

Agonist Specificity



	IL-1β
S/B	6.1
EC ₅₀ (ng/mL)	0.88

Antagonist Specificity



	IL-1β	Anakinra	Adalimumab
S/B	5.5	4.2	--
EC ₅₀ / IC ₅₀ (ng/mL)	0.84	302	--

Benefits for “Ready-to-Use” Bioassay Kits

Functional response based on drug MOA

Verified and Qualified with innovator’s marketed drug

Simple protocol; Rapid results

Specific and Sensitive assay

Highly reproducible

Readily Implement with Optimized kit

- Frozen ready-to-assay cells
- Bioassay Detection Reagents
- Cell Plating Reagent
- Dilution Buffer
- Control Agonist
- Tissue Culture-Treated Plates

For More Info, Questions or Technical Support



Web:

[Cell-Based Bioassays for Biologics](#)

Technical Support

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