



PathHunter[®] Sargramostim Bioassay

Qualified with Leukine[®]

93-1078Y3-00111 (2-Plate Kit)

93-1078Y3-00112 (10-Plate Kit)

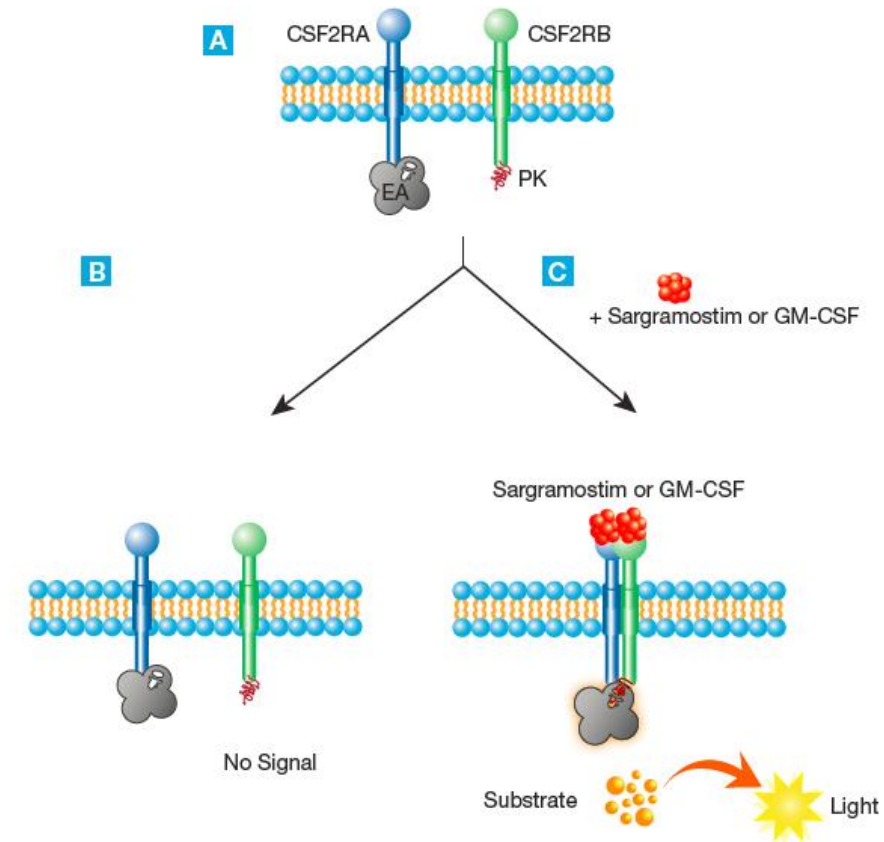
OUR EXPERTISE
IN YOUR HANDS.
DISCOVER
CONFIDENTLY.

For Treatment of Neutropenia and Myeloid Reconstitution after Bone Marrow Transplantation

Mechanism of Action

- Sargramostim is a recombinant form of the naturally occurring granulocyte macrophage-colony stimulating factor, GM-CSF
- It binds to and activates the GM-CSF receptor, a heterodimer comprised of CSF2RA and CSF2RB, leading to formation of white blood cells (e.g. granulocytes and macrophages) that detect and destroy harmful bacteria and fungi.

Assay Principle



PathHunter® Sargramostim Bioassay Kit

Kit Components

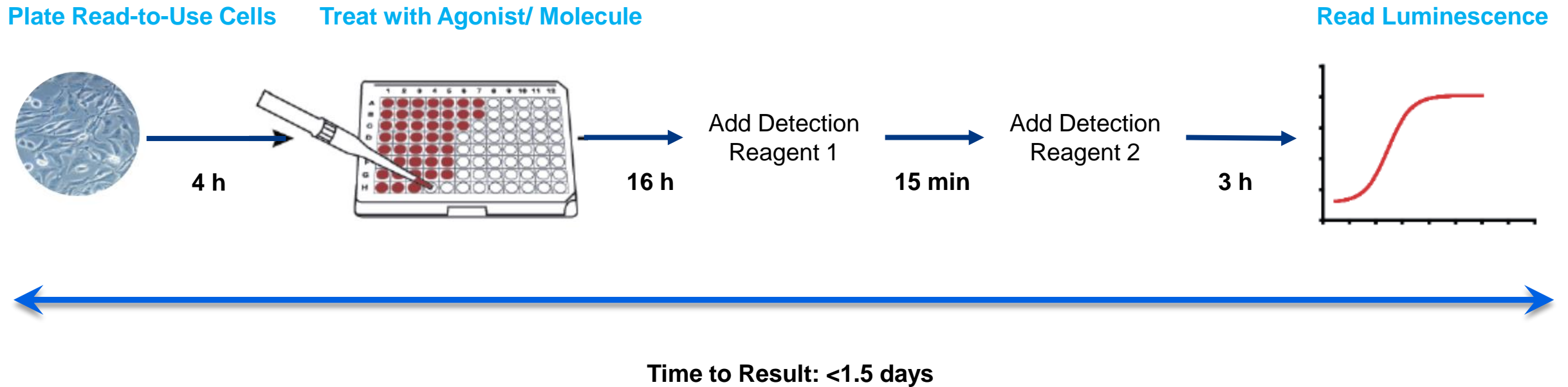
List of Components	93-1078Y3-00111	93-1078Y3-00112
PathHunter U2OS CSF2RB/CSF2RA Bioassay Cells	2 vials	10 vials
PathHunter Bioassay Detection Kit		
Detection Reagent 1 (mL)	2	10
Detection Reagent 2 (mL)	8	40
AssayComplete™ Cell Plating Reagent 22	1 X 100 mL	2 X 100 mL
AssayComplete Dilution Buffer B5*	1 X 100 mL	1 X 100 mL
Control Agonist (GM-CSF)	1 vial	1 vial
96-Well Clear-Bottom TC Treated, Sterile Plates w/Lid	2 plates	10 plates

Sample Qualification data

Sargramostim Bioassay Qualification

Bioassay Workflow

Simple, Homogenous and Rapid Protocol



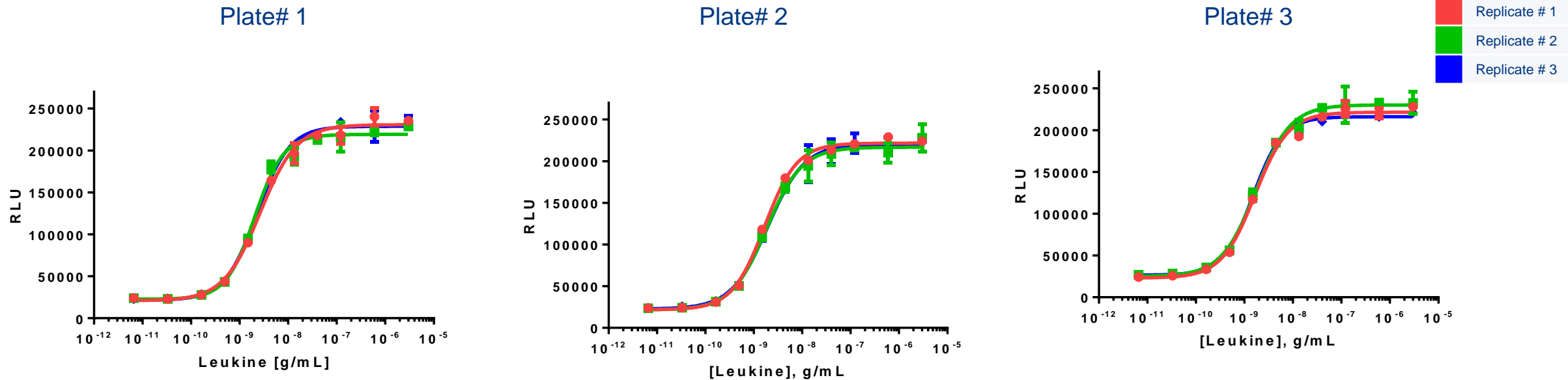
Sargramostim Bioassay Qualification with Leukine®

Assay Parameters Assessed

- % CV between 8 full plate DRCs
- Plate uniformity: EC_{80} 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

Sargramostim Bioassay Qualification with Leukine®

Plate to plate variability: 3 plates with full-plate DRC - Same Day



Parameter	R1	R2	R3
S/B	10.3	9.6	9.8
Hill Slope	1.154	1.458	1.295
EC ₅₀ (ng/mL)	2.65	2.05	2.28

Parameter	R1	R2	R3
S/B	9.4	9.4	9.0
Hill Slope	1.29	1.192	1.155
EC ₅₀ (ng/mL)	1.67	1.84	1.94

Parameter	R1	R2	R3
S/B	9.2	8.8	8.3
Hill Slope	1.259	1.215	1.403
EC ₅₀ (ng/mL)	1.65	1.73	1.5

Assay Robustness

Repeatability and Intermediate Precision (Inter-Plate)

Plate	Sample	S/B	S/B (%RSD)	EC ₅₀ (ng/mL)	Mean EC ₅₀ (ng/mL)	EC ₅₀ (%RSD)
1	R1	10.3	3.6	2.65	2.33	12.9
	R2	9.6		2.05		
	R3	9.8		2.28		
2	R1	9.4	2.5	1.67	1.82	7.7
	R2	9.4		1.84		
	R3	9.0		1.94		
3	R1	9.2	5.1	1.65	1.62	7.1
	R2	8.8		1.73		
	R3	8.3		1.50		

Intermediate Precision (Inter-Day)

Day	EC ₅₀ , ng/mL	Mean EC ₅₀ , ng/mL	%RSD, EC ₅₀
1	2.15	1.96	9.2
2	1.80		
3	1.92		

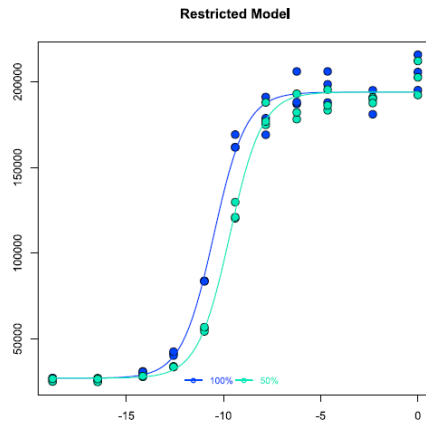
Intermediate Precision (Inter-Plate): 9.2%
Intermediate Precision (Inter-Day): 9.2%

Sargramostim Bioassay Qualification with Leukine®

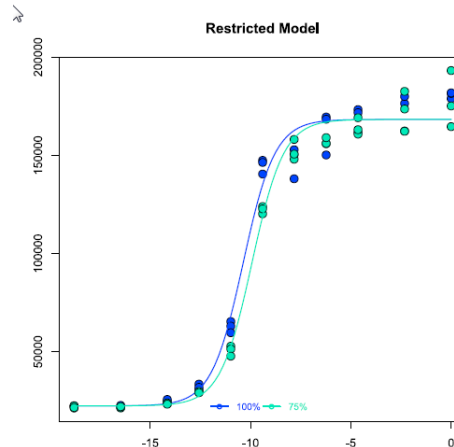
Parallelism and Potency Estimation (PLA):

Analyst 1

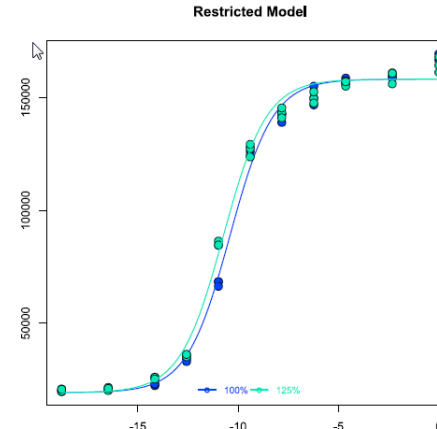
50%



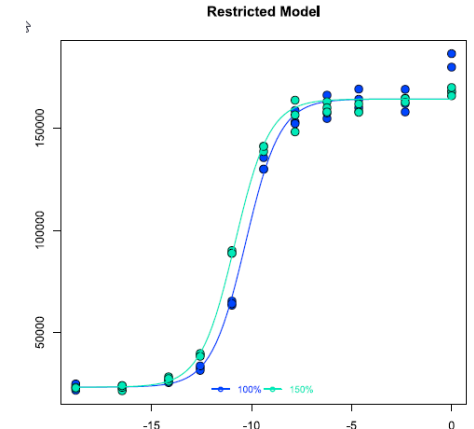
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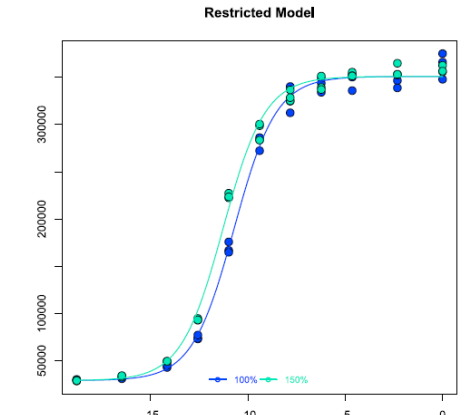
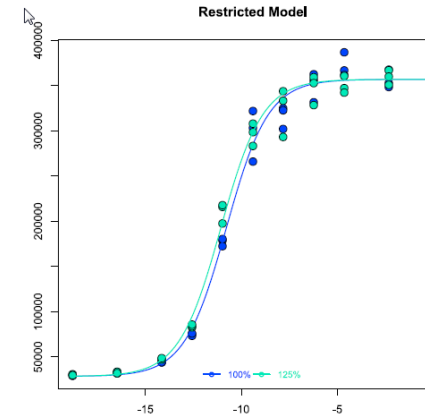
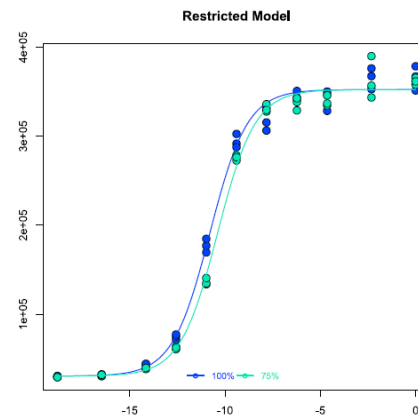
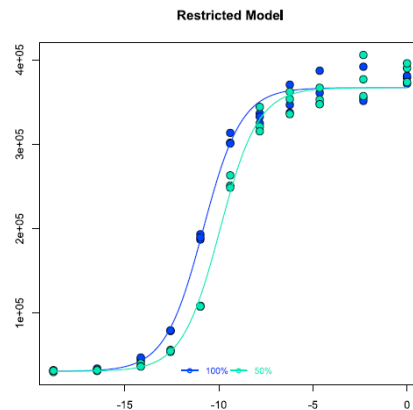
125%



150%



Analyst 2

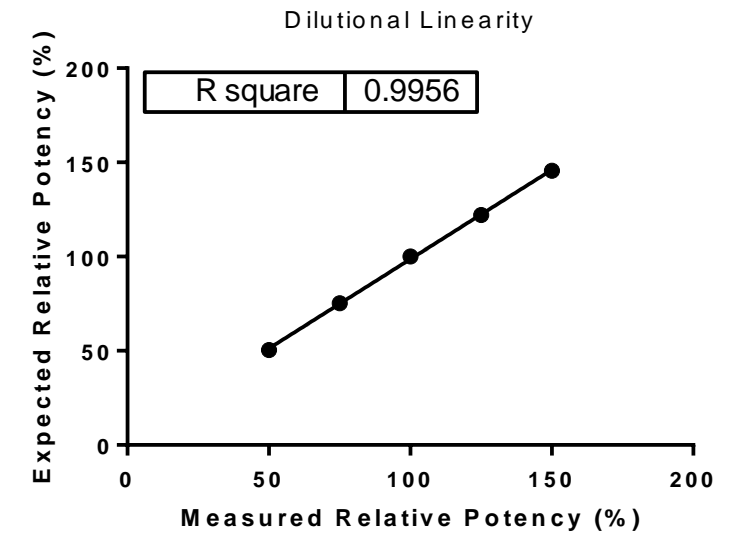


● Reference ● Sample

Sargramostim Bioassay Qualification with Leukine®

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

Expected RP (%)	Exp No.	Analyst No.	Measured RP (%)	Average RP (%)	% RSD	% Recovery
150	1	1	140.8	145.6	2.46	97.1
	2	1	145			
	3	1	148.5			
	4	2	148.2			
125	1	1	118.5	122.1	2.29	97.7
	2	1	122.5			
	3	1	125.3			
	4	2	122.1			
75	1	1	77.4	75.3	2.11	100.4
	2	1	74.4			
	3	1	73.8			
	4	2	75.6			
50	1	1	47.5	50.5	5.03	101
	2	1	50.5			
	3	1	50.2			
	4	2	53.7			



Accuracy: 99.1%

Precision: 3%

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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