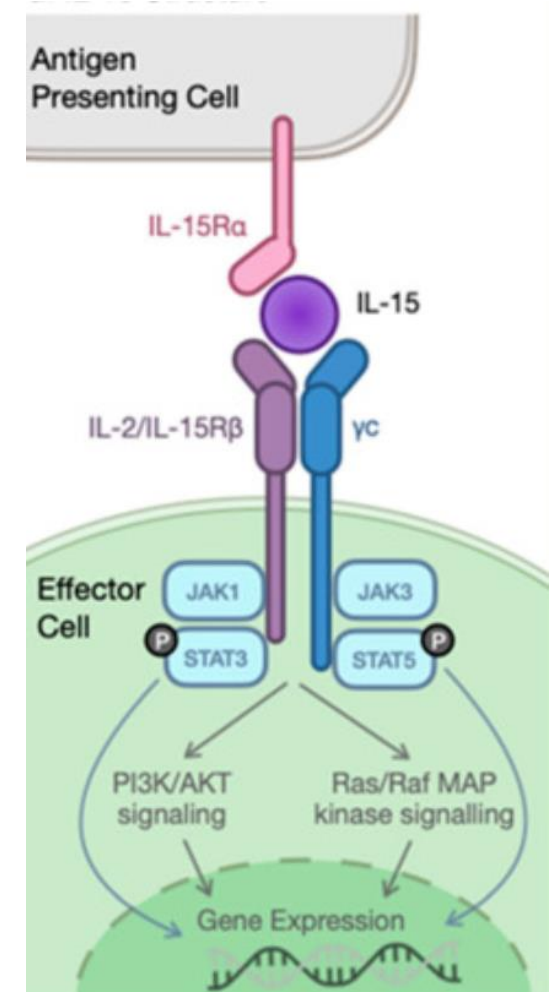




PathHunter IL-15 Bioassay (Reporter) Qualification Data

Background / Assay Concept: IL-15 Bioassay (Reporter): For Quantitation of IL-15

- IL-15 binds to the alpha subunit of the IL15 receptor (IL-15R α) with high affinity.
- IL-15 also binds to the beta and gamma chains (also referred to as the $\beta\gamma$ complex) of the IL-2 receptor, but not to the alpha subunit of the IL2 receptor.
- In vivo, IL-15 is presented in trans to the beta and gamma chains of the IL-2 receptor expressed on T cells and NK cells as a complex with the IL-15R α
- Engagement of the $\beta\gamma$ complex by IL-15 leads to activation of STAT5 signaling through JAK3
- REH cells endogenously express IL2RB, but heterologous expression of IL2RB and IL2RG was employed to improve surface expression of each
- This assay quantifies IL-15-mediated activation of a STAT5-responsive transcriptional reporter that produces an ED-tagged reporter protein.



Kit Component (Volume)	# Supplied
PathHunter REH IL-15 Signaling Reporter Bioassay Cells (1.2 x 10⁶ cells in 0.1 mL per vial)	10 [‡]
AssayComplete™ Cell Plating 0 Reagent (100 mL per bottle)	2*
Protein Dilution Buffer (50 mL bottle)	2*
PathHunter PL/PK Detection Kit (mL per bottle)	
EA Reagent (20 mL)	1
Lysis Buffer (20 mL)	1
Substrate Reagent (80 mL)	1
96-Well White, Clear Flat-Bottom, TC-Treated, Sterile Plates with Lid	10

*Reagent bottles are provided with the indicated volume plus 20% overage to allow for generation of smaller aliquots.

‡Excess cells are provided in each vial; leftover cells may be discarded once plating is complete

Optimized IL-15 Dose Curve and Plate Layout Used For Assay Qualification

Dilution Scheme

Concentration, ng/mL	Diln Factor
50	
12.5	4
3.13	4
1.25	2.5
0.5	2.5
0.20	2.5
0.08	2.5
0.032	2.5
0.013	2.5
0.003	4
0.0008	4

Representative Dose Curve (100% NC)

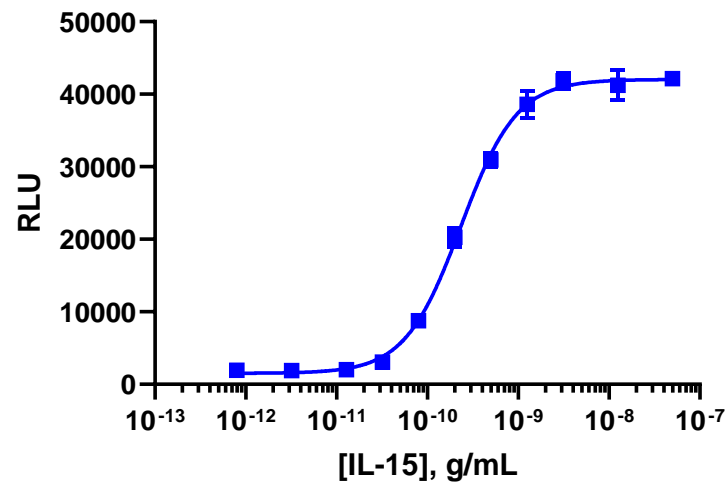
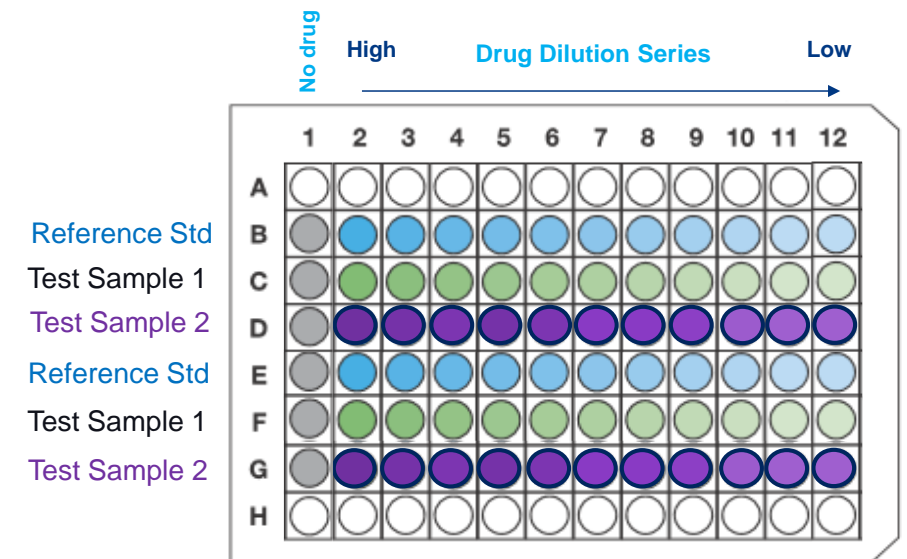


Plate Layout



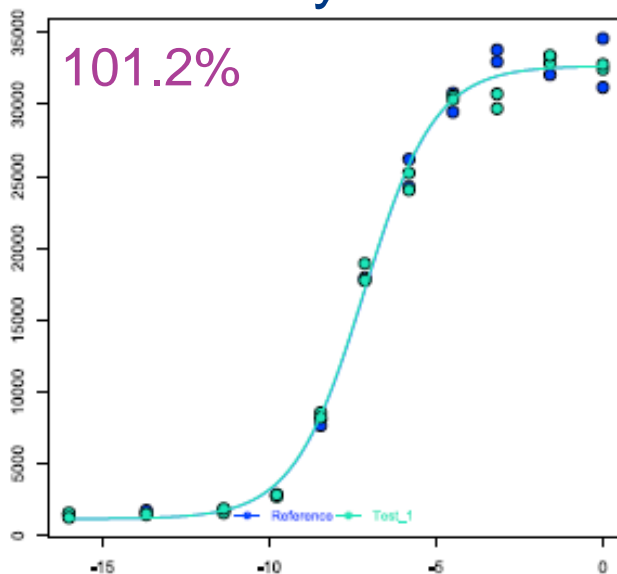
Day	Nominal Concentrations	Analyst
1	100% x 6 (Repeatability)	1
2	200%, 150%, 125%, 100%, 75%, 50%	1
2/3	150%, 100%, 50%, 70%	2
3	150%, 125%, 100%, 75%, 50%	1
4	200%, 125%, 75%, 140%	2
4	150%, 125%, 75%, 50%, 100%	1
5	150%, 125%, 75%, 50%	1
5	150%, 125%, 75%, 50%	2
6	200%, 100%	1 or 2

- Established minimum system and sample suitability (SST) criteria for S/B, replicate precision, curve shape and parallelism / linearity for restricted model in PLA 3.0

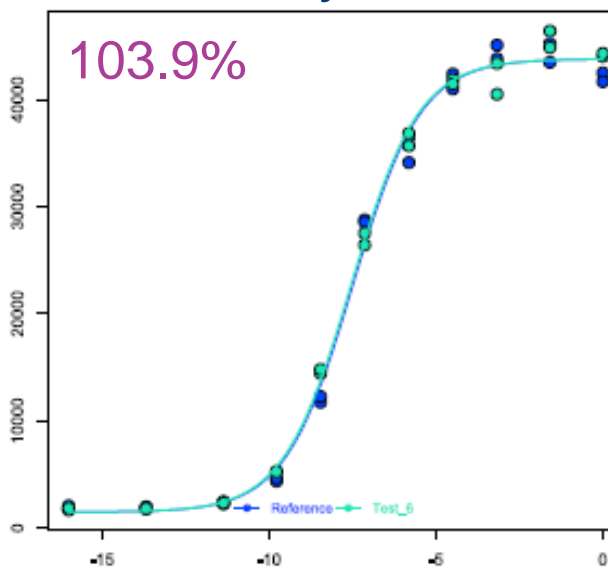
- Repeatability: performed 6 runs of 100% NC by single analyst
- Intermediate precision: evaluated 5 nominal concentrations (NC) over a range of 50%-150%
 - n ≥ 6 for each NC
 - Each performed by 2 analysts
 - Multiple days
- Dilutional linearity evaluated over range of 50%-200%, incorporating additional NC's for 70%, 140% and 200%
- 99% success rate (1 plate failed SST for replicate precision)

Day-to Day Repeatability (100% NC; Single Analyst)

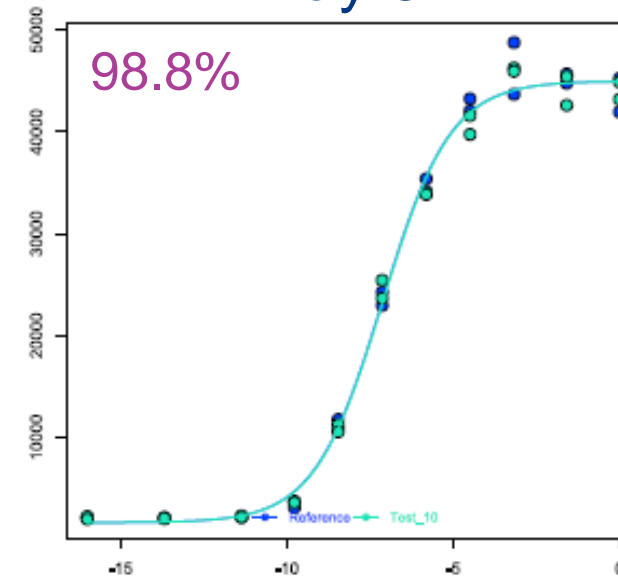
Day 1



Day 2



Day 3

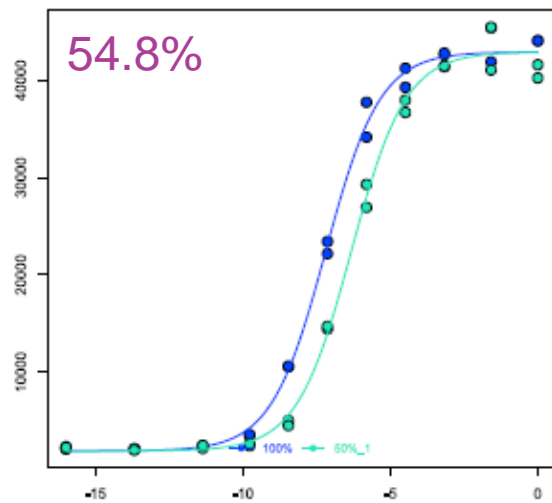


Day	S/B	HillSlope	R ²	Max % Diff	Measured RP, %	Potency Range, 95% CI, %	Relative CI, %	Width of Relative CI, %	Linearity / Parallelism
1	30.3	1.3372	0.9932	23%	101.2%	0.85923 - 1.1918	84.91% - 117.78%	32.87%	Pass / Pass
2	32.8	1.3119	0.9942	10%	103.9%	1.35500 - 1.95365	83.28% - 120.08%	36.79%	Pass / Pass
3	28.5	1.3745	0.9946	12%	98.8%	0.84320 - 1.15729	85.36% - 117.15%	31.80%	Pass / Pass

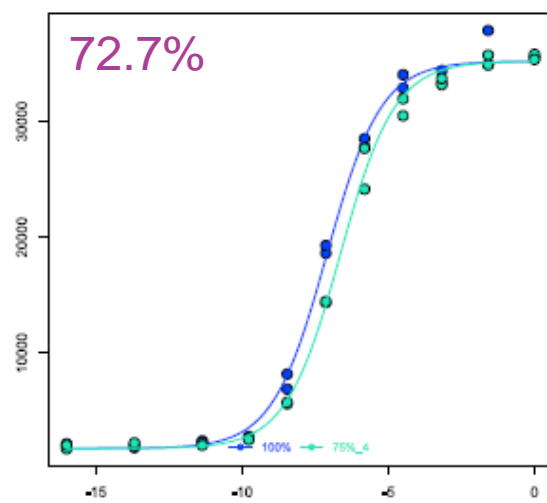
Representative Relative Potency Data (4 Nominal Concentrations) (Analyst 1 vs Analyst 2)

Analyst 1

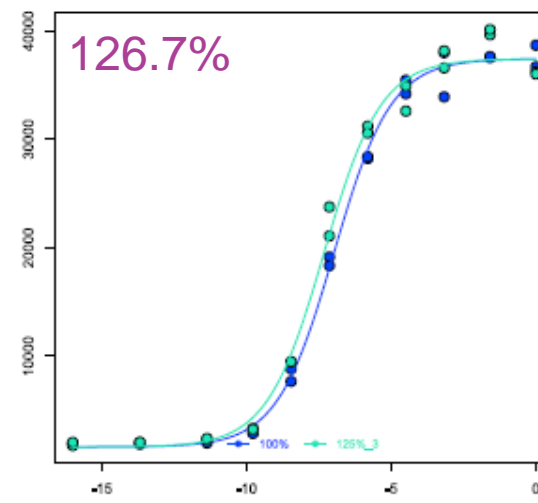
50%



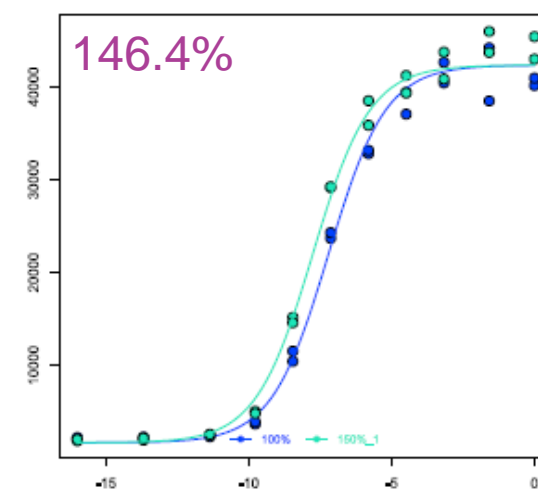
75%



125%

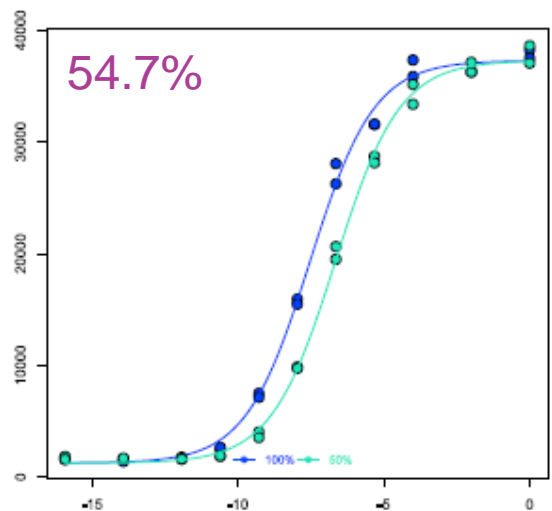


150%

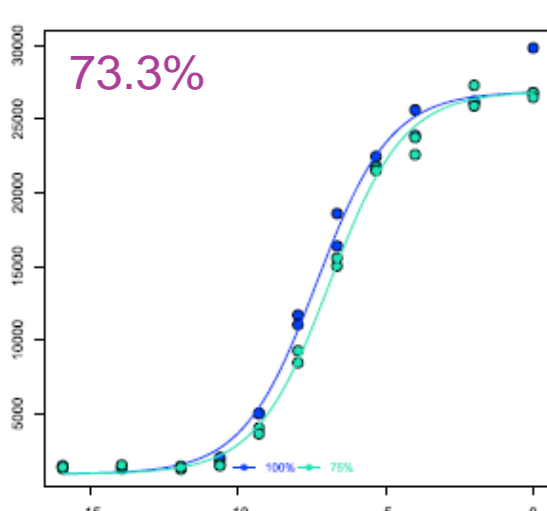


Analyst 2

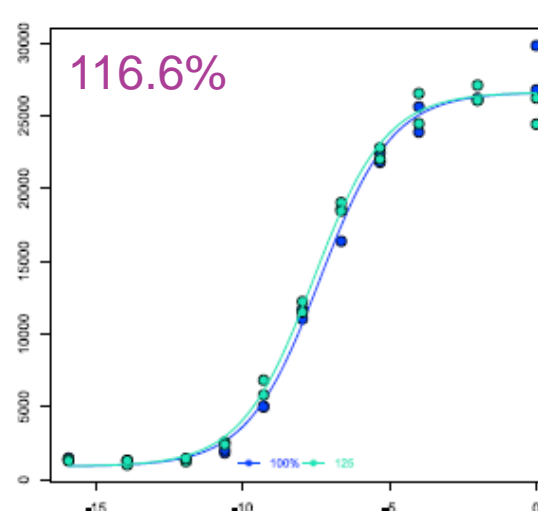
54.7%



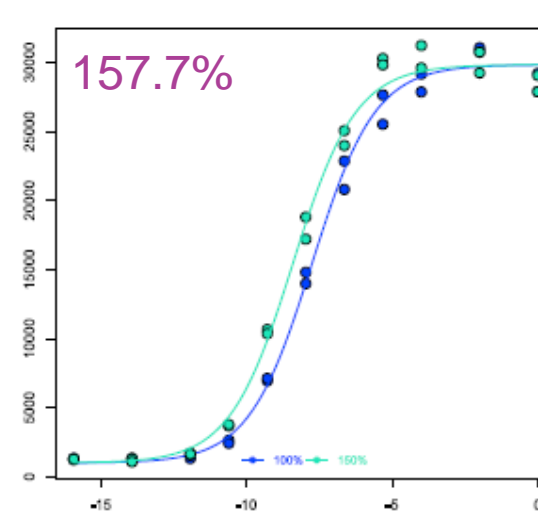
73.3%



116.6%



157.7%



Assessment of Repeatability with 100% NC (Single Analyst)

Expected RP, %	Exp # / Plate #	Analyst	Measured RP, %	Potency Range, 95% CI, %	Geometric Mean RP, %	% GCV	% Recovery
100	1/1	1	103.9	0.91641 - 1.17901	100.5	7.2	101
	1/2	1	98.8	0.79609 - 1.22401			
	1/3	1	90.6	0.76670 - 1.06991			
	1/4	1	100.2	0.81672 - 1.22848			
	1/5	1	98.8	0.84320 - 1.15729			
	1/6	1	112.1	0.93290 - 1.34589			

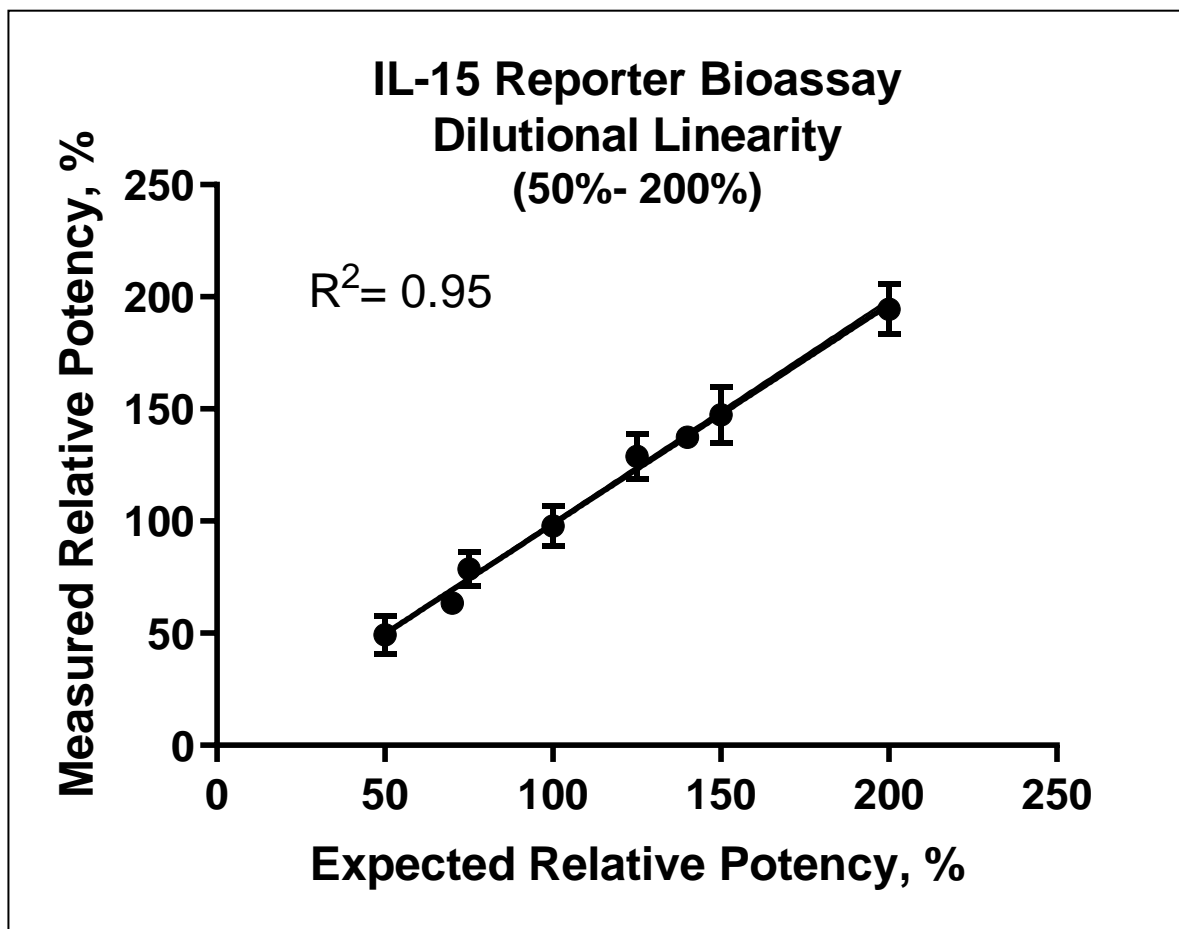
Assessment of Accuracy and Intermediate Precision with 100% NC (Two Analysts)

Expected RP, %	Exp #	Analyst	Measured RP, %	Potency Range, 95% CI, %	Geometric Mean RP, %	% GCV	Relative Bias, %
100	1	1	101.2	0.85923 - 1.19187	98.7	9.6	-1.3
	2	1	112.8	0.98197 - 1.29599			
	3	1	87.7	0.73794 - 1.04220			
	4	1	103.9	0.91641 - 1.17901			
	5	1	97.6	0.81153 - 1.17271			
	6	2	90.8	0.72293 - 1.14011			

Accuracy and Intermediate Precision for Remaining NC's

Expected RP	Expt #	Analyst	Measured RP, %	Potency Range, 95% CI	Relative Recovery	GM RP, %	GCV, %	Relative Bias, %
200%	1	1	202.4	1.72349 - 2.37728	101.2	194.4	5.9	-2.8
	2	2	186.7	1.58310 - 2.20286	93.4			
150%	1	1	146.4	1.18445 - 1.81018	97.6	146.8	9.2	-2.1
	2	1	143.2	1.17727 - 1.74105	95.4			
	3	1	123.9	1.05550 - 1.45425	82.6			
	4	1	151.7	1.23593 - 1.86307	101.2			
	5	1	145.1	1.24908 - 1.68537	96.7			
	6	2	157.7	1.31769 - 1.88729	105.1			
	7	2	163.2	1.41817 - 1.87913	108.8			
125%	1	1	138.8	1.22228 - 1.57601	110.3	128.4	8.3	2.72
	2	1	134.9	1.12600 - 1.61521	107.9			
	3	1	126.7	1.05884 - 1.51711	101.4			
	4	1	123.7	1.03525 - 1.47886	98.9			
	5	1	117.9	0.90579 - 1.53518	94.3			
	6	2	142.8	1.16073 - 1.75647	114.2			
	7	2	116.6	0.91793 - 1.48007	93.3			
75%	1	1	86.1	0.75062 - 0.98753	114.8	78.3	10.1	4.4
	2	1	69.7	0.54911 - 0.88395	92.9			
	3	1	86.7	0.76059 - 0.98915	115.6			
	4	1	72.7	0.62426 - 0.84598	96.9			
	5	2	83	0.68637 - 1.00422	110.7			
	6	2	73.3	0.59878 - 0.89800	97.7			
50%	1	1	54.8	0.45836 - 0.65501	109.6	52	6.4	4
	2	1	52.3	0.41323 - 0.66083	104.5			
	3	1	48.8	0.40668 - 0.58597	97.6			
	4	1	51.6	0.42862 - 0.62161	103.2			
	5	1	47.1	0.39882 - 0.55563	94.1			
	6	2	55.4	0.46235 - 0.66359	110.8			
	7	2	54.7	0.48811 - 0.61272	109.4			

- Good accuracy with relative recovery between 80-120% for all levels tested
- Intermediate precision: $\leq 10.1\%$ GCV at all levels tested
- Slight negative relative bias at high concentrations (150% and above), with slightly positive relative bias at low end of assay range



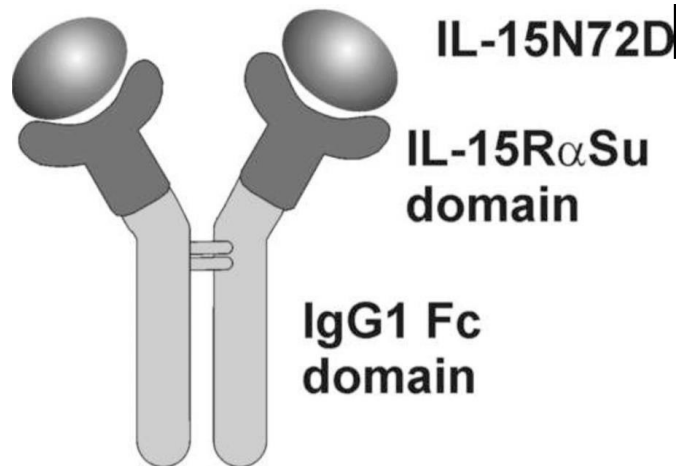
Parameter	Value	Specification
Accuracy (Average % Recovery)	101.5%	100% +/- 20%
Repeatability, %GCV	7.2%	≤20%
Intermediate Precision, % GCV	≤10.1%	≤20%
Linearity (R^2)	0.95	≥0.95

Excellent accuracy, repeatability, intermediate precision, and good dilutional linearity

Response to Recombinant Human IL-15R α & IL-15 Fusion Protein vs Soluble IL-15 in IL-15 Bioassay (Reporter)

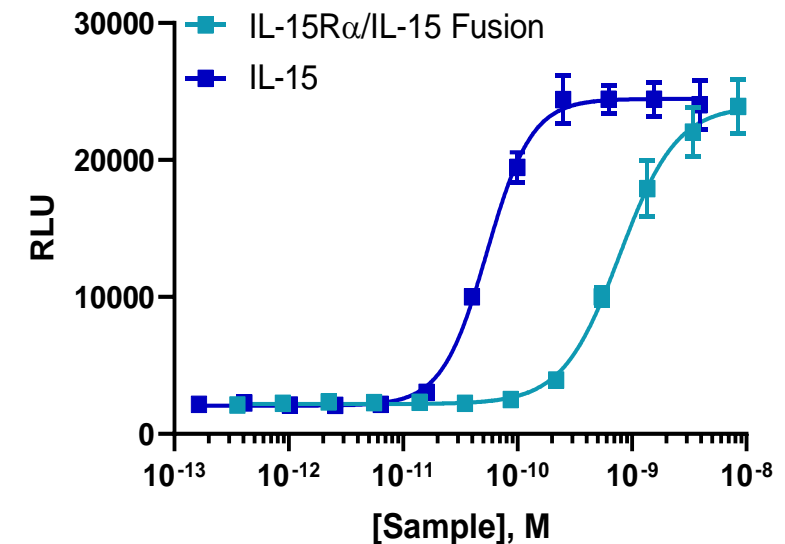
Engineered IL-15: IL15R α 'super' agonist

Rh IL-15R α / IL-15 fusion protein
Abeomics (Cat #: 32-8662)



- In vivo, IL-15 is presented in trans by the IL-15 receptor (IL-15R) alpha-chain to the IL-15R $\beta\gamma\epsilon$ complex displayed on the surface of T cells and natural killer (NK) cells.
- Fusion of IL-15 to the sushi domain of IL-15R α is an approach several companies are taking to improve biological activity of IL-15 therapeutics
- Engineering mutations in IL-15 (e.g. N72D) further improves biological activity relative to W/T IL-15

PathHunter IL-15 Bioassay (Reporter)



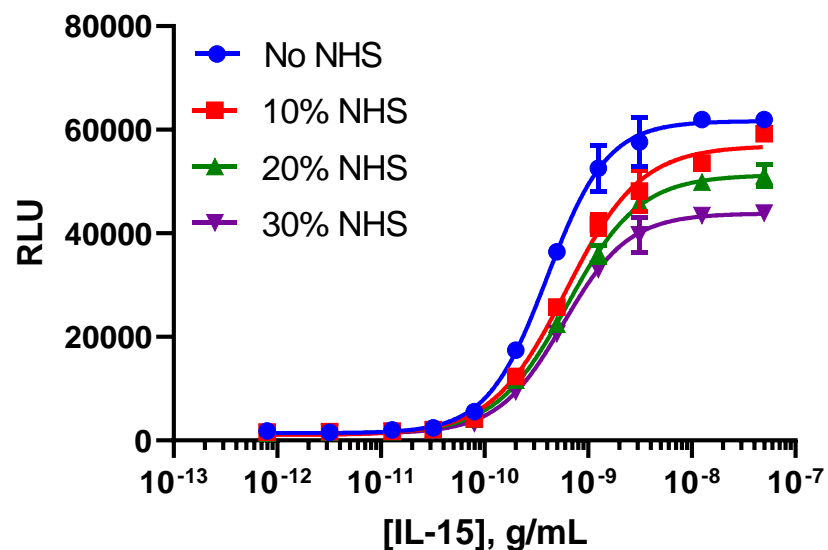
Sample	S/B	HillSlope	EC ₅₀ , nM
IL-15	14.7	2.213	0.054
IL15Ra / IL-15 Fusion	14.7	1.740	0.78

Reporter bioassay ~13-fold more sensitive to IL-15 than dimer bioassay; same rank order of two proteins in each assay

Performance of the PathHunter IL-15 Bioassay (Reporter) is Minimally Impacted by Normal Human Serum (NHS)

PH IL-15 Bioassay (Reporter)

Part #: 93-1178Y055 Lot #: 23C07MN



Sample	S/B	HillSlope	EC ₅₀ , pg/mL
No NHS	34.2	1.494	402
10% NHS	35.9	1.210	615
20% NHS	35.2	1.253	633
30% NHS	32.7	1.382	575