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KILR® Raji ADCC Bioassay

Qualified with Rituximab

97-1012Y026-00169 (2-Plate Kit)

95-1012Y026-00170 (10-Plate Kit)

OUR EXPERTISE
IN YOUR HANDS.
DISCOVER
CONFIDENTLY.

KILR Raji ADCC Bioassay Kit

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Kit Components

List of Components	97-1012Y026-00169 (2 plate Kit)	97-1012Y026-00170 (10 plate Kit)
KILR Raji Bioassay Cells (1 x 10^6 cells /100 µL /vial)	2 vials	10 vials
AssayComplete™ Cell Plating 39 Reagent (Bottle)	1 x 100 mL	1 x 500 mL
KILR Detection Kit		
Detection Reagent 1 (Bottle)	1 x 17 mL	1 x 85 mL
Detection Reagent 2 (Bottle)	1 x 5 mL	1 x 25 mL
Detection Reagent 3 (Bottle)	1 x 5 mL	1 x 25 mL
Total Lysis Control (Bottle)	1 x 0.5 mL	1 x 2.5 mL
96-Well White, Flat-Bottom, TC-Treated, Sterile Plates with Lid	2	10

KILR Raji ADCC Bioassay Kit

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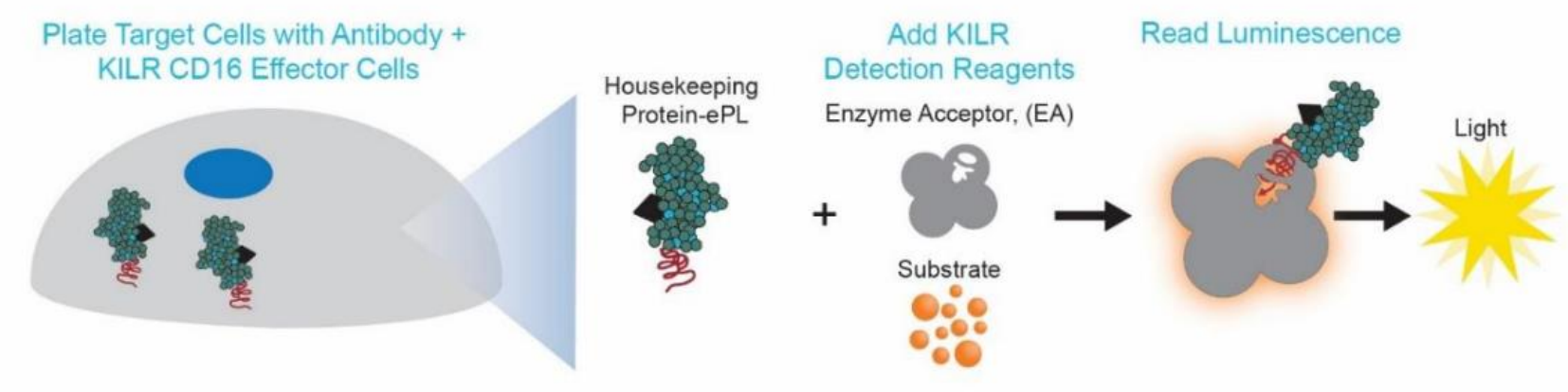
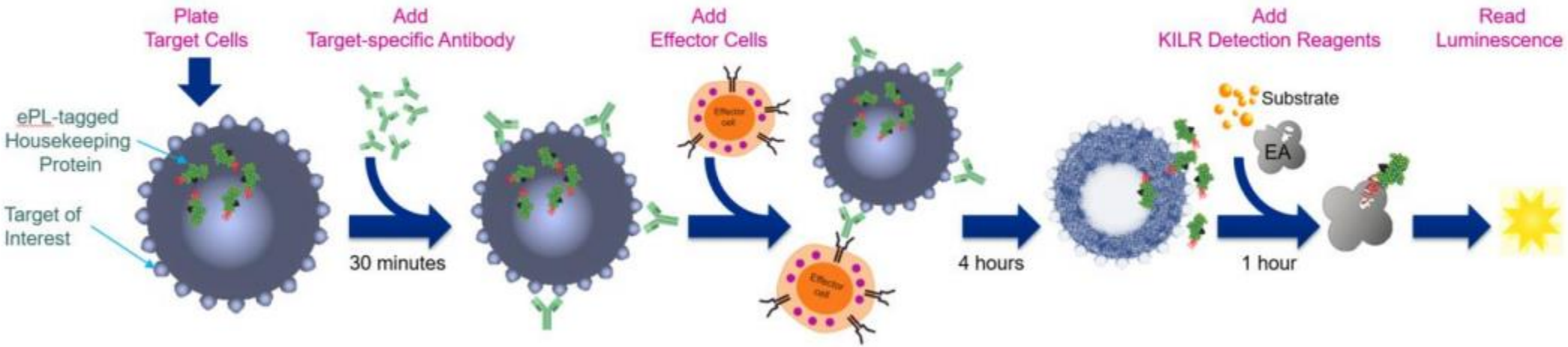
Technology Principle



Assay Principle



Sample data

KILR Raji ADCC Bioassay Qualification

Assay Conditions

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Assay Conditions	
Vial Density, Target Cells (KILR Raji Bioassay Cells)	1 x 10^6 / 100 µL
Vial Density, Effector Cells (KILR CD16 Effector Cells)	20 x 10^6 / 1,000 µL
Target Cell density per well (KILR Raji cells)	5,000
Antibody incubation time (and temp)	30 min @ 37°C
Antibody top dose	8 ng/mL
Incubation time with Effector cells, hours (and temp)	4 h @ 37°C
Recommended Effector : Target (E:T) Ratio	10:1
Density of effector cells in culture before assay	1 x 10^6 cells / mL
Total number of effector cells/well	10 x 5K cells/well = 50K cells/well
KILR Detection reagent incubation time	1 h @ R/T in the dark

Reagents Used in Qualification Study

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Item	Description	Vendor	Part #
Plate Type	96-Well White, Flat-Bottom, TC-Treated, Sterile Plates with Lid	DiscoverX	92-0027
Bioassay Cells	KILR Raji Bioassay Cells	DiscoverX	97-1012Y026
Assay Medium	AssayComplete Cell Plating 39 Reagent (CP39)	DiscoverX	93-0563R39
Detection Reagent Kit	KILR Detection Reagent	DiscoverX	97-0001M
Effector Cells	KILR CD16 Effector Cells	DiscoverX	97-0007-01
Effector Cell Culture Medium	Assay Complete Cell Culture Kit-117	DiscoverX	92-3117G
Ligand (for Cell Culture)	Recombinant Human IL-2, Cell Culture Grade	DiscoverX	92-1331
Control Antibody	Rituximab (Afucosylated hIgG1 anti-CD20 antibody)	Invivogen	hcd20-mab13

Optimized Dose Curve and Plate Layout

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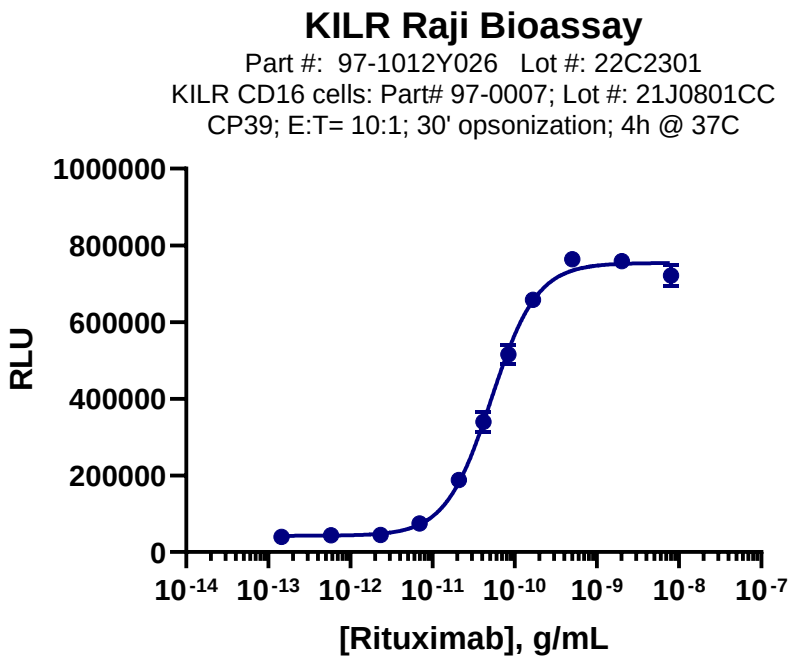
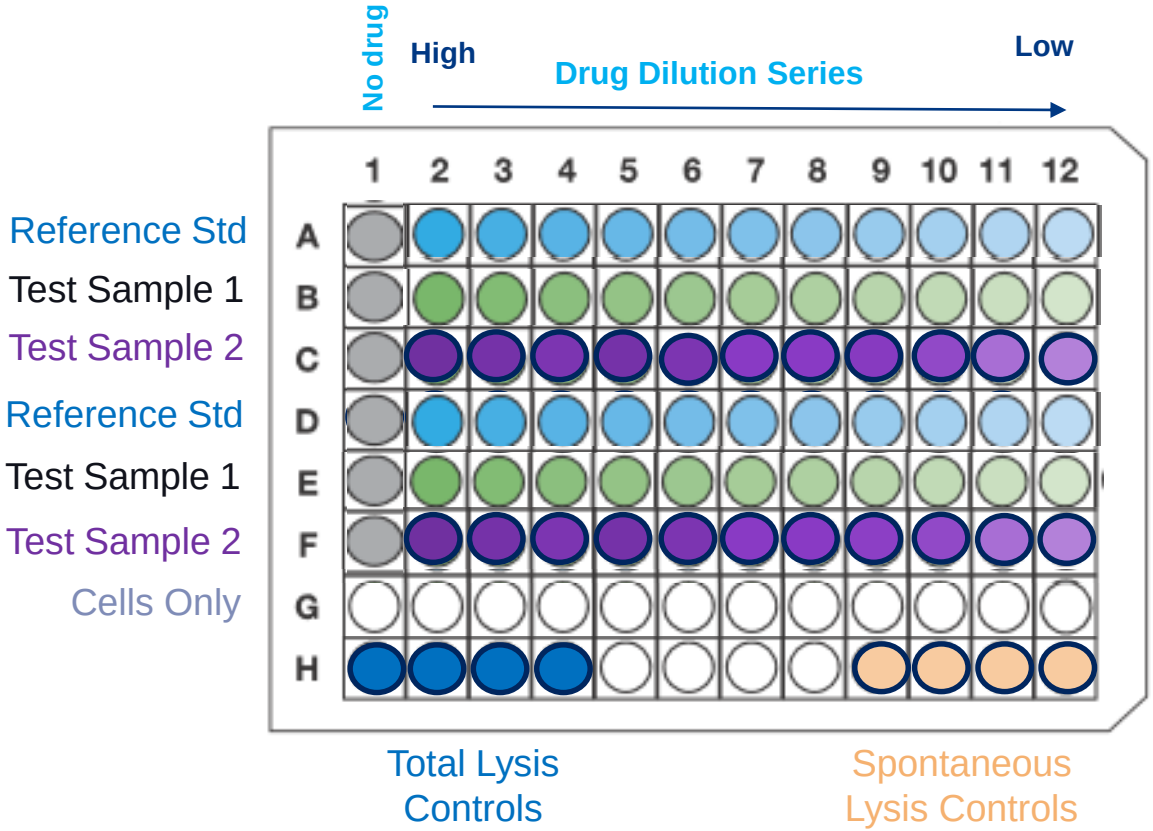


S/B	HillSlope	EC_{50}, pg/mL
17.8	1.563	51.6

Dilution Series

Conc, ng/mL	Dilution Factor
8	
2	4
0.5	4
0167	3
0.083	2
0.042	2
0.021	2
0.0069	3
0.0023	3
0.00058	4
0.00014	4

Plate Layout: Duplicates / dose



Qualification Study Design

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

- Evaluated 5 nominal concentrations (NC) over a range of 50%-150%
  - (n ≥4 for each NC)
- Repeatability: 4 runs of 100% NC by single analyst
- Intermediate precision incorporates:
  - 2 analysts
  - Multiple days
  - 2 lots of bioassay target cells
  - 3 lots of KILR CD16 effectors

System and Sample Suitability Criteria Used For Qualification

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Parameter Description	Parameter Value	System Suitability, Y/N	Sample Suitability, Y/N
Curve Shape (Minimum of 2 data points in upper and lower asymptotes and 3 data points in linear portion of curve)	Pass	Y	Y
Max % Difference for replicates	FIO	N	N
Minimum signal-to-background (A/D)	S/B ≥ 7	Y	Y
F-tests for linearity and parallelism (in PLA 3.0), using 95% confidence interval	Pass	N	Y
Relative confidence interval around relative potency estimation	FIO	N	N
The percent difference between the (A-D) of the unconstrained reference curve and the (A-D) of the unconstrained test sample curve.	Percent Difference (A-D): ≤15%	N	Y
The percent difference between the slopes (B) of the unconstrained reference curve and the unconstrained test sample curve	Percent Difference in Slopes (B): ≤30%	N	Y
Percent Recovery (for individual nominal concentration)	Within 80-120% of expected value	N	Y

System and Sample Suitability Data

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Date	Analyst	Effect or Lot	Plate	Sample	Nominal Potency	Observed Potency	% Recovery	Curve Shape (Restricted Model)	Max % Difference	A/D Ratio (S/B)	Relative Confidence Interval, %	% Difference (A-D)	% Difference Slope	F-Test, Linearity	F- Test, Parallelism	Pass / Fail
5-May	1	A	1	Std	Std			Pass	16%	10.6						
				1	100%	82.0%	82%	Pass	11%	10.2	43.0%	2%	3%	Passed	Passed	Pass
5-May	1	A	2	Std	Std			Pass	24%	17.8						
				1	150%	156.0%	104%	Pass	19%	20.2	32.6%	1%	1%	Passed	Passed	Pass
				2	50%	47.8%	96%	Pass	25%	19.8	24.0%	3%	6%	Passed	Passed	Pass
9-May	1	A	1	Std	Std			Pass	16%	14.8						
				1	125%	130.6%	104%	Pass	8%	13.1	31.7%	2%	2%	Passed	Passed	Pass
				2	75%	70.4%	94%	Pass	19%	14.8	31.0%	0%	0%	Passed	Passed	Pass
10-May	1	B	1	Std	Std			Pass	35%	17.3						
				1	150%	191.0%	127%	Pass	22%	20	45.3%	1%	10%	Passed	Passed	Fail
				2	50%	45.8%	92%	Pass	21%	19.2	42.3%	5%	12%	Passed	Passed	Pass
11-May	1	B	1	Std	Std			Pass	22%	16.5						
				1	125%	131.2%	105%	Pass	21%	16.7	45.1%	4%	1%	Passed	Passed	Pass
				2	75%	76.6%	102%	Pass	19%	17.6	40.0%	1%	6%	Passed	Passed	Pass
17-May	1	A	1	Std	Std			Pass	16%	25.9						
				1	150%	179.4%	120%	Pass	38%	22.7	25.4%	1%	13%	Passed	Passed	Pass
				2	50%	40.9%	82%	Pass	25%	23.9	22.5%	5%	12%	Passed	Passed	Pass
18-May	1	A	1	Std	Std			Pass	23%	15.9						
				1	125%	126.0%	101%	Pass	20%	15.3	29.6%	1%	13%	Passed	Passed	Pass
				2	75%	77.3%	103%	Pass	26%	15.5	36.2%	1%	5%	Passed	Passed	Pass
19-May	1	A	1	Std	Std			Pass	14%	19.8						
				1	100%	116.0%	116%	Pass	17%	17.7	31.4%	4%	3%	Passed	Passed	Pass
				2	100%	124.1%	124%	Pass	33%	16.5	34.9%	2%	1%	Passed	Passed	Fail
19-May	1	A	2	Std	Std			Pass	22%	26.4						
				1	100%	112.7%	113%	Pass	11%	22.9	18.4%	1%	7%	Passed	Passed	Pass
				2	100%	98.4%	98%	Pass	14%	19.5	26.2%	6%	8%	Passed	Passed	Pass
20-May	2	A	1	Std	Std			Pass	9%	12.8						
				1	100%	112.1%	112%	Pass	16%	12.6	20.6%	1%	4%	Passed	Passed	Pass
24-May	2	C	1	Std	Std			Pass	8%	7.1						
				1	50%	58.4%	117%	Pass	9%	7.4	28.0%	4%	12%	Passed	Passed	Pass
				2	150%	166.0%	111%	Pass	17%	7.8	25.2%	4%	8%	Passed	Passed	Pass
25-May	2	C	1	Std	Std			*Failed*	13%	6.2						
				1	75%	84.7%	113%	*Failed*	18%	5.3	33.3%	8%	7%	Passed	Passed	Fail
26-May	2	C	1	Std	Std			*Failed*	20%	2.7						
				1	125%	139.9%	112%	*Failed*	20%	2.7	66.8%	1%	4%	Passed	Passed	Fail
27-Jul	1	D	1	Std	Std			Pass	17%	25						
				1	150%	177.0%	118%	Pass	17%	34.3	29.1%	7%	5%	Passed	Passed	Pass
28-Jul	2	D	1	Std	Std			Pass	12%	10.3						
				1	125%	121.4%	97%	Pass	7%	9.8	24.7%	4%	7%	Passed	Passed	Pass
28-Jul	2	D	2	Std	Std			Pass	12%	10.3						
				1	75%	71.1%	95%	Pass	12%	10.2	23.1%	0%	6%	Passed	Passed	Pass
29-Jul	1	D	1	Std	Std			Pass	19%	20.9						
				1	100%	117.8%	118%	Pass	14%	23.7	30.9%	1%	6%	Passed	Passed	Pass
29-Jul	2	D	1	Std	Std			Pass	17%	10.2						
				1	150%	154.3%	103%	Pass	12%	10.7	28.0%	1%	2%	Passed	Passed	Pass
				2	50%	49.4%	99%	Pass	22%	10.7		0%	3%	Passed	Passed	Pass

- 28 total samples (nominal concentrations) tested during qualification:
  - 24 / 28 samples passed system and sample acceptance criteria= 86% success rate
  - Failed assays due to % recovery outside range (>120%) or low S/B (A/D ratio)

Representative Relative Potency Data (Analyst 1 vs Analyst 2)

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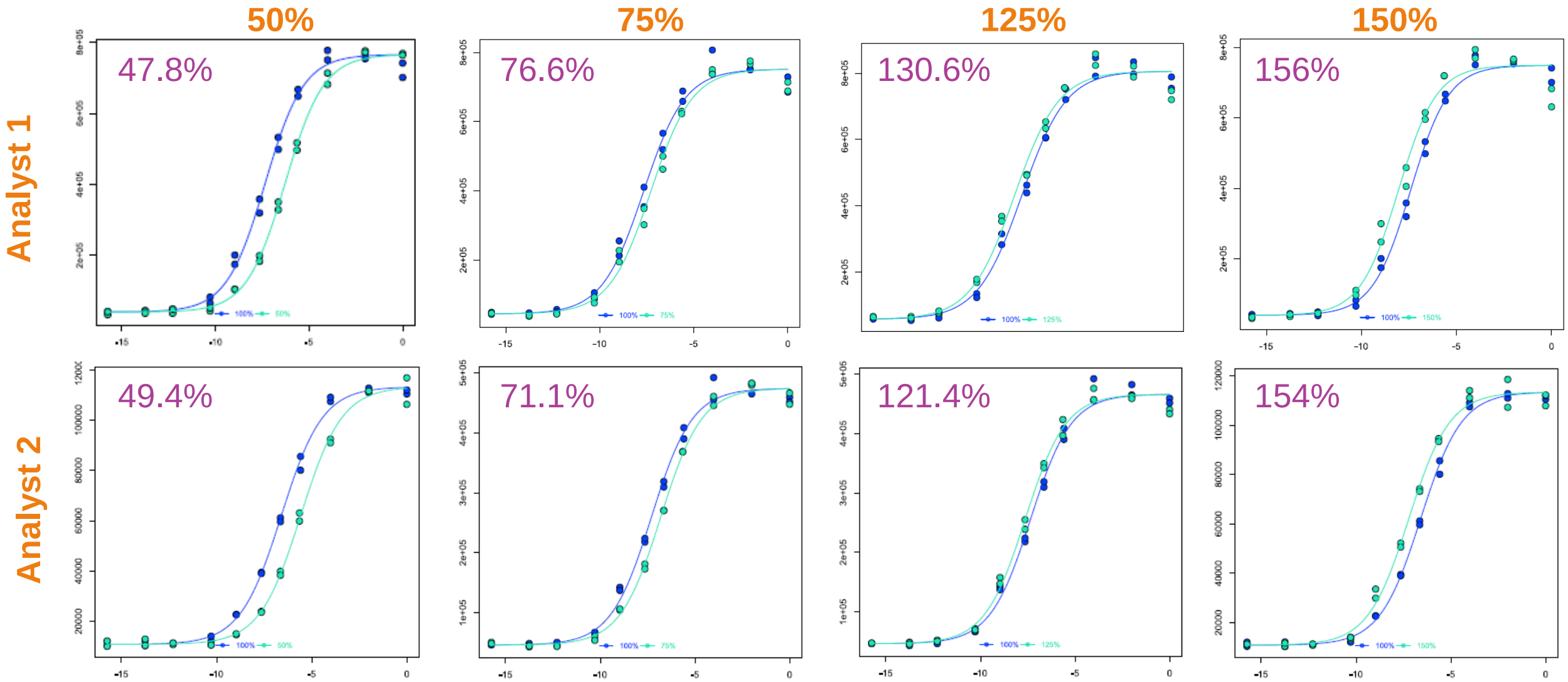

Qualification Data with Rituximab Demonstrates Suitability of KILR® Raji Bioassay for Relative Potency Assays

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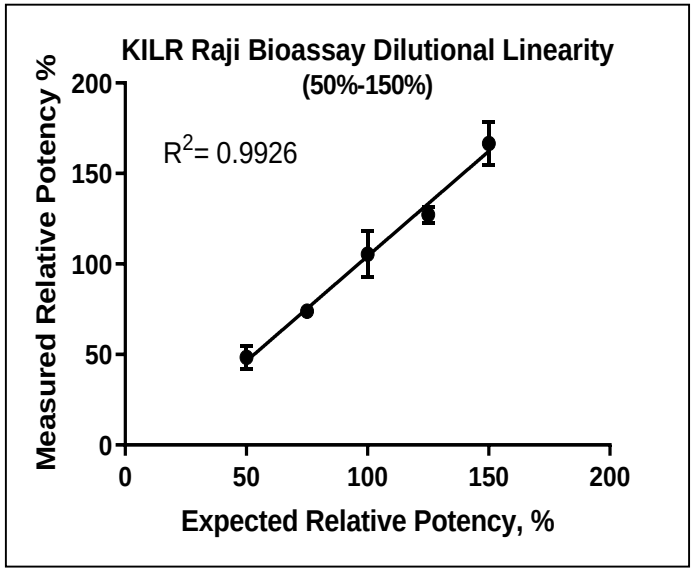
Nominal RP, %	Analyst	Observed RP, %	Average RP, %	% RSD	Average % Recovery
150	1	156	166.6	7.0	111.1%
	1	179.4			
	1	177.4			
	2	166			
	2	154.3			
`125	1	130.6	127.3	3.6	101.8%
	1	131.2			
	1	126			
	2	121.4			
100	1	82	106.5	13.0	106.5%
	1	116			
	1	112.7			
	1	98.4			
	1	117.8			
	2	112.2			
75	1	70.4	73.9	4.9	98.5%
	1	76.6			
	1	77.3			
	2	71.1			
50	1	47.8	48.5	13.2	96.9%
	1	45.8			
	1	40.9			
	2	58.4			
	2	49.4			



Parameter	Value	Specification
Accuracy (Average % Recovery)	103%	100% +/- 20%
Repeatability	14.2%	≤20%
Intermediate Precision	≤13.2%	≤20%
Linearity (R^2)	0.9926	≥0.95

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

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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