



Implementing MOA-Reflective ADCC & ADCP Cytotoxicity Assays Using Ready-to-Use KILR[®] Target & Effector Cells from Screening to Lot Release

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Eurofins DiscoverX

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R&D & Manufacturing
San Francisco Bay Area, California (HQ)
St. Charles, Missouri
Poitiers, France

10+

Druggable
target
classes

1500+

Stable cell line and
membrane preps

20+

Core patents

2000+

Publications across multiple
applications

55+

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MOA-based
bioassays

Validated

>30 Billion Data Points
screened in assay services with
same assays

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CRO Partners**

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enable global CROs

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**Dedicated
Scientific Support**

Experienced team providing
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**20+ Successful
Assay Transfers**

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For potency, stability and NAb
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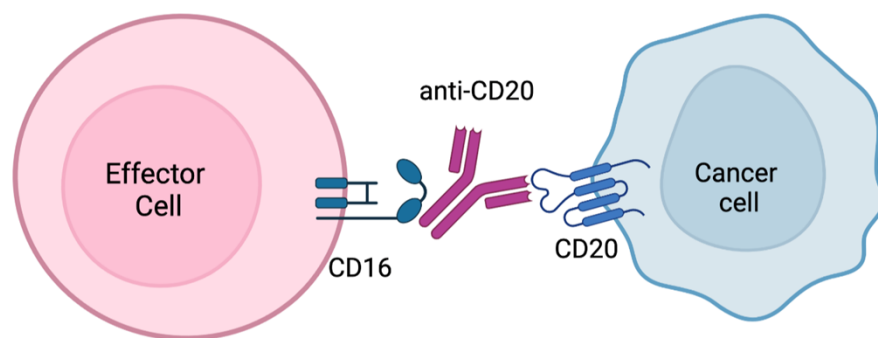
MOA = Mechanism-of-Action; ICH = International Council for Harmonization; CRO = Contract Research Organizations; NAb = Neutralizing Antibody

Cell-Mediated Cytotoxicity – A Rapidly Developing Area for Cancer Therapeutics

Therapeutic mAbs are the fastest growing class of biological therapeutics, and over 100 mAbs are already approved by the FDA

Many of these therapeutic antibodies work by activating antibody-dependent cell-mediated cytotoxicity (ADCC) or other effector-mediated functions (ADCP, CDC, T-cell Redirection)

Starting from Phase I and even earlier, functional assays to assess effector function (e.g. ADCC) should be performed to evaluate therapeutic antibody potency and in other cases for safety



Therapeutic antibodies to induce ADCC approved in the US

Antibody	Type	Target
Rituximab/other similar anti-CD20	Chimeric IgG1	CD20
Trastuzumab	Humanized IgG1	HER2
Alemtuzumab	Humanized IgG1	CD52
Cetuximab	Chimeric IgG1	EGFR
Ofatumumab	Human IgG1	CD20
Pertuzumab	Humanized IgG1	HER2
Dinutuximab	Chimeric IgG1	GD2

Challenges with Existing Cytotoxicity Assays

What scientists face....

Radioactivity or fluorescent dye-based are inherently leaky – produce less robust and high background

Low assay throughput restricts screening and characterization efficiency

Surrogate assays fail to reflect the MOA
“*Predictive*” and not “*Reflective*”

High donor-to-donor variability associated with effector cells

- KILR® assays are *non-radioactive and not leaky*

Little to no background

- KILR cytotoxicity assays are *high-throughput compatible (384-well)*

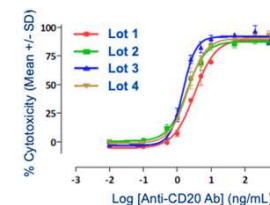
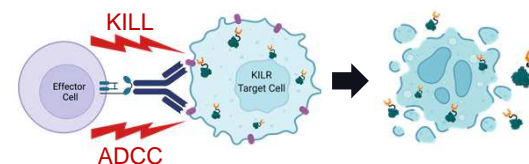
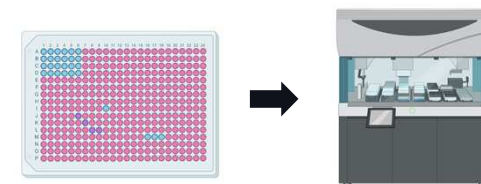
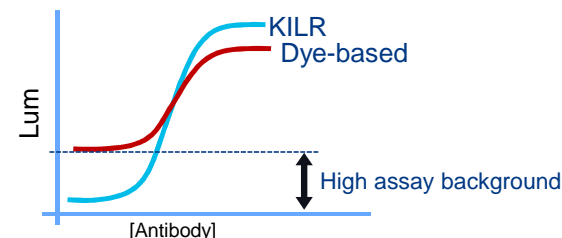
Ease of implementation from screening to lot release

- KILR assays are *end-point assays and specifically measure target cell killing*

Physiologically-reflective of TRUE MOA

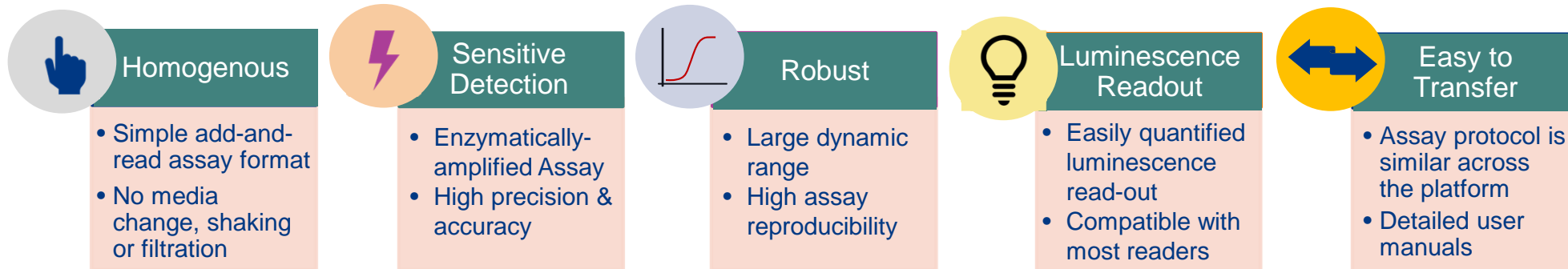
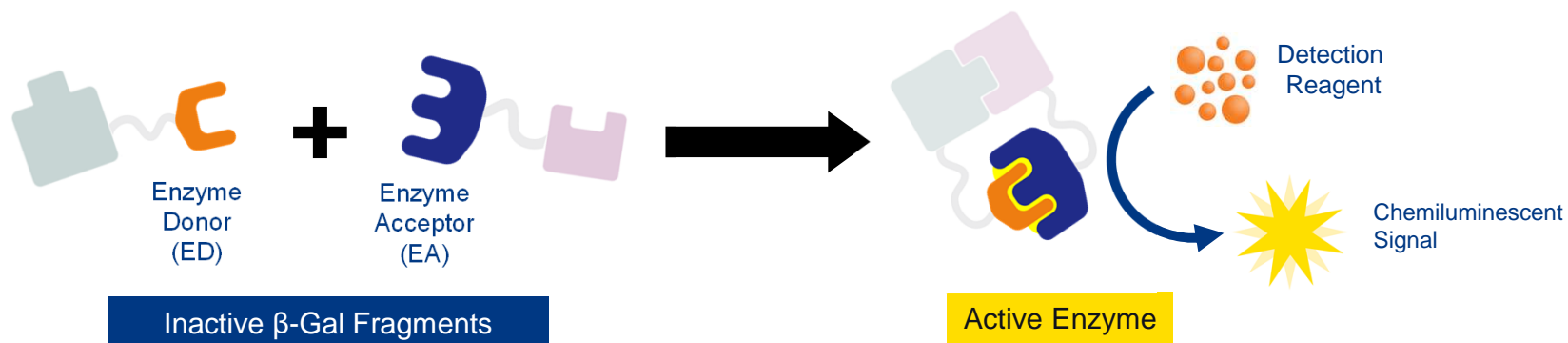
- KILR CD16 Effector Cells are *single donor-derived*

Eliminate donor variability



Enzyme Fragment Complementation (EFC) Technology

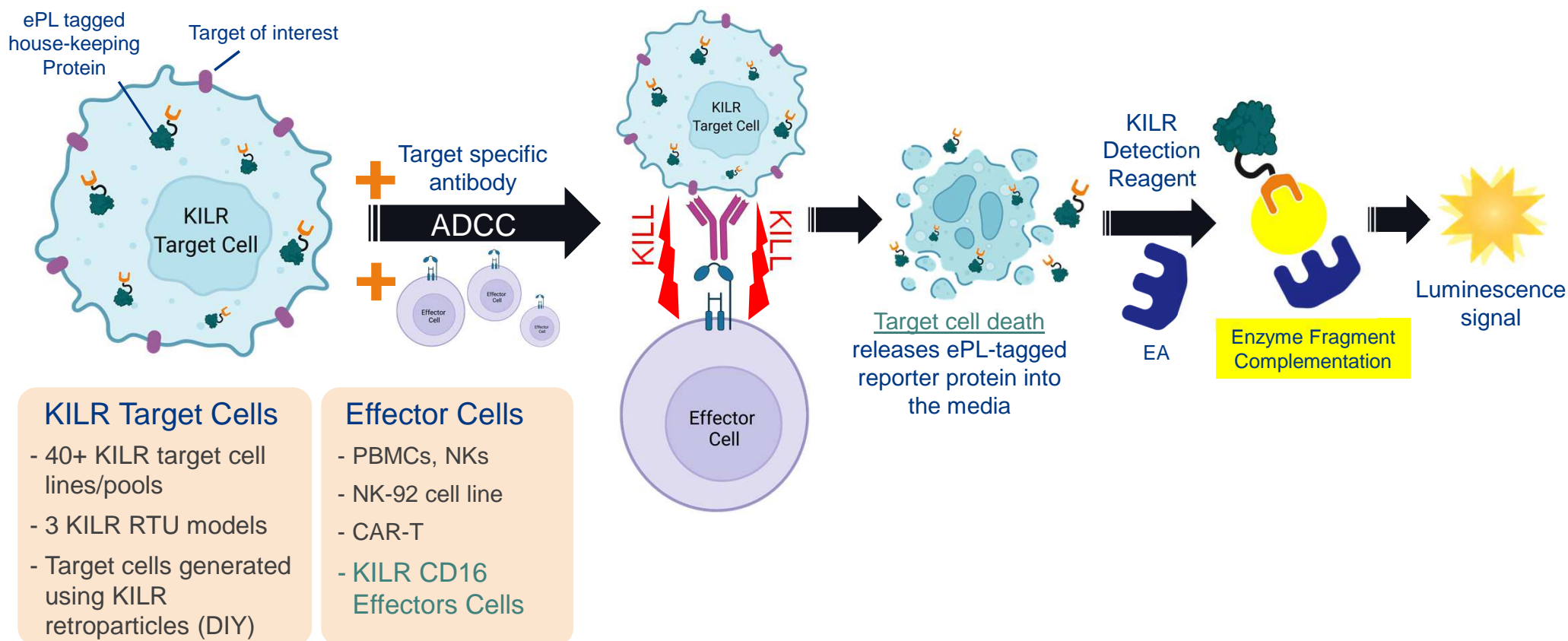
Enabling Technologies with a Flexible Platform based on a Split β -Galactosidase Enzyme



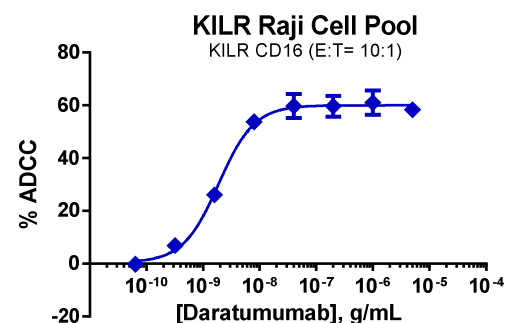
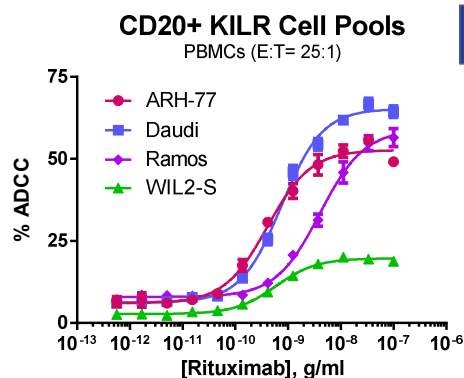
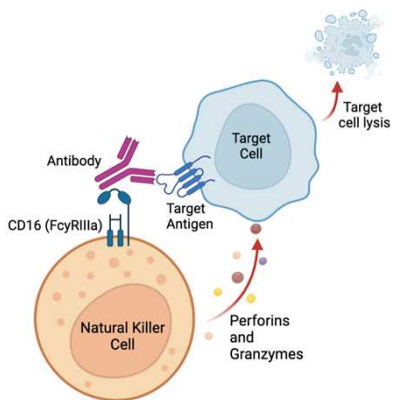
KILR® Cytotoxicity Assay Overview

ADCC Example

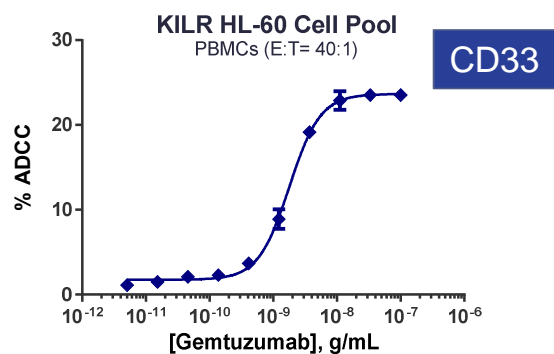
An easy-to-use assay to specifically measure target cell death



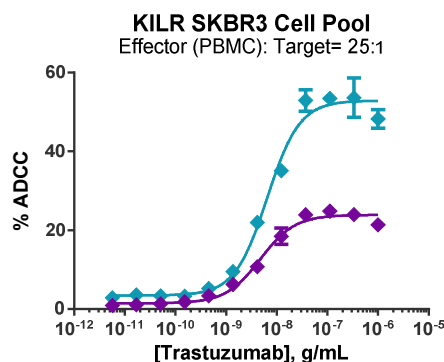
KILR[®] Assay ADCC Demonstrated Using a Variety of Antibodies, Antigens, and Cell Types



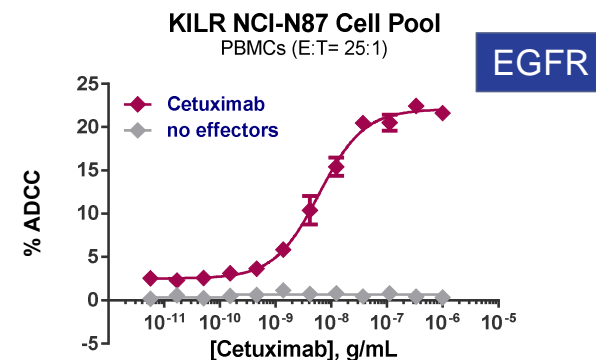
Sample	E _{Max}	EC ₅₀ , ng/mL
Daratumumab	60%	1.89



Sample	E _{Max}	EC ₅₀ , ng/mL
Gemtuzumab	24%	1.78



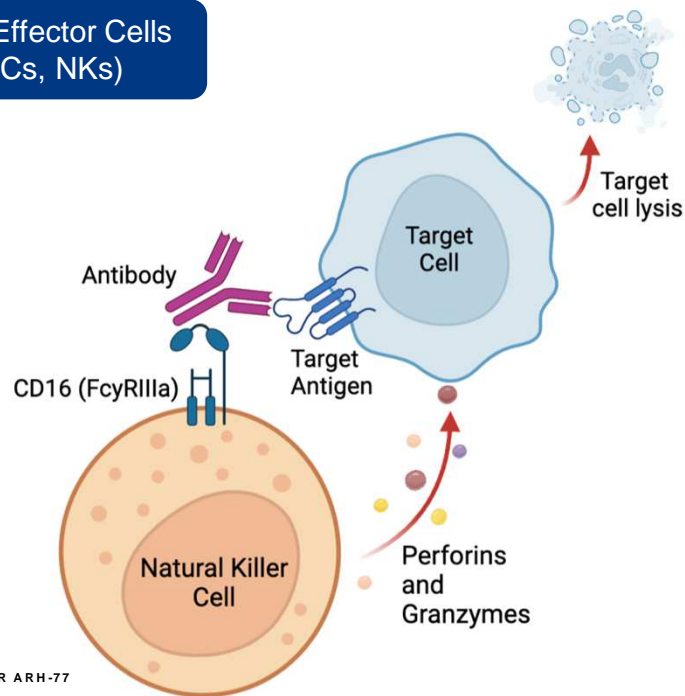
Sample	E _{Max}	EC ₅₀ , ng/mL
Trastuzumab (5K, 10K)	53.6%, 24.8%	4.74, 6.44



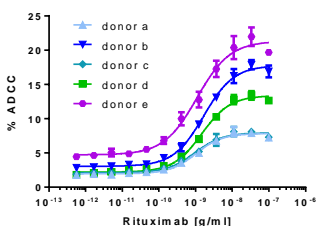
Sample	E _{Max}	EC ₅₀ , ng/mL
Cetuximab	22%	6.04

Eliminating Donor Variability in ADCC Assays Using KILR® CD16 Effector Cells

Isolated Effector Cells (PBMCs, NKs)



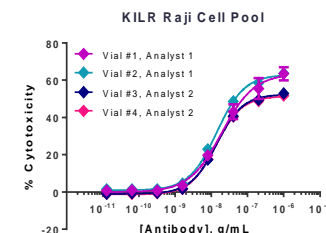
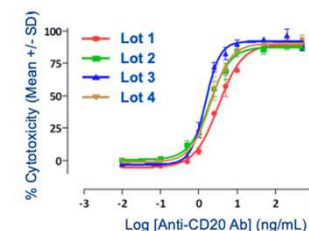
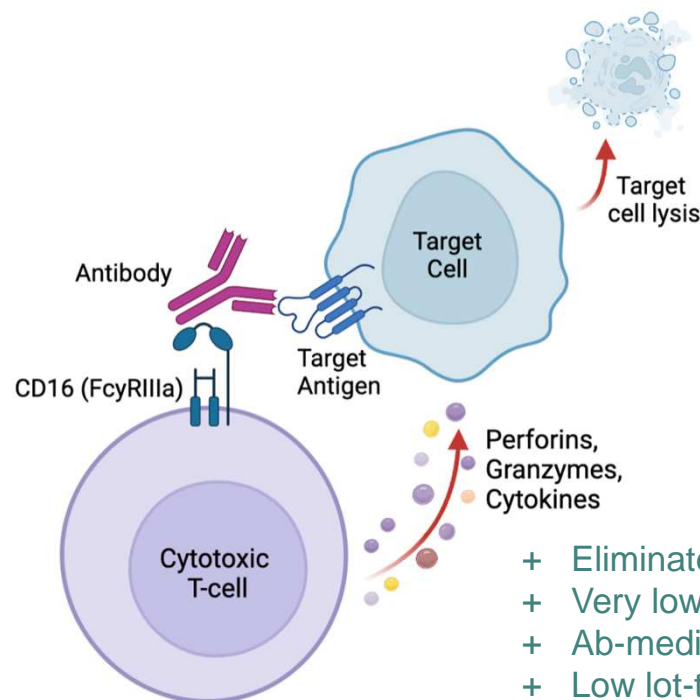
ADCC with KILR ARH-77



- High donor variability
- Heterogenous population (PBMCs)
- High lot-to-lot variability
- High background killing (NK cells)

KILR CD16 Effector Cells

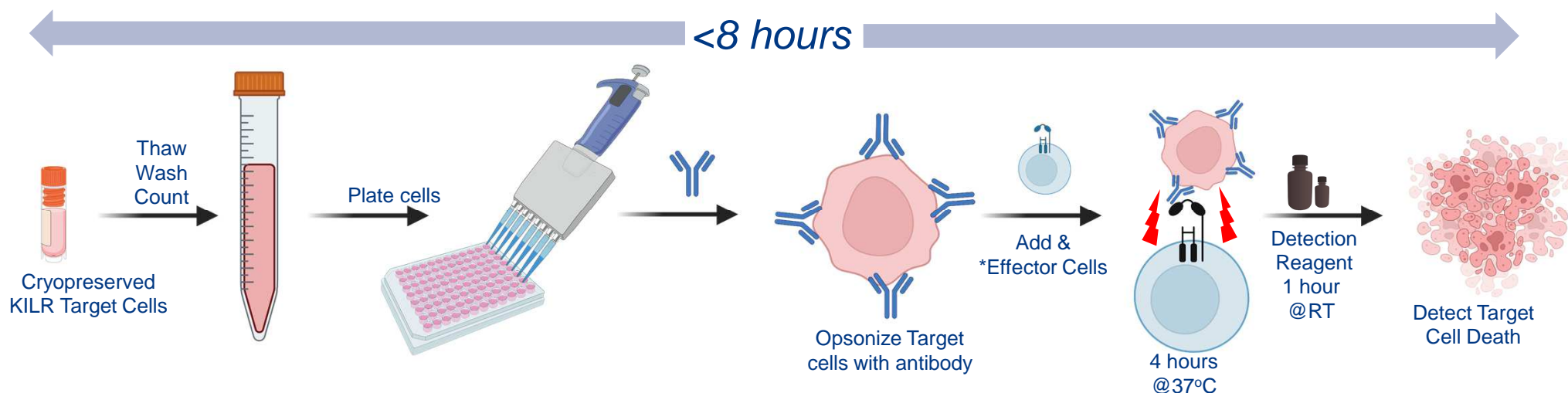
Single donor-derived Cytotoxic T-cells engineered to express CD16 receptor



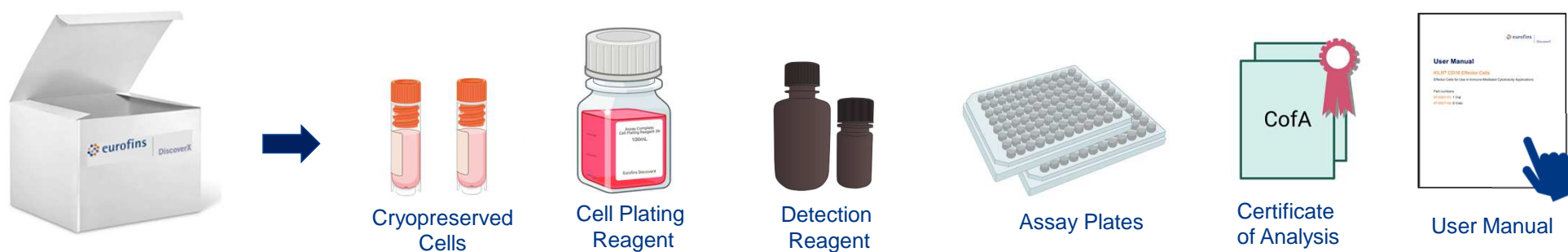
- + Eliminates donor variability
- + Very low background killing
- + Ab-mediated rapid killing kinetics
- + Low lot-to-lot variability

Ready-to Use Format

Easy-to-Use KILR[®] Raji ADCC Bioassay Kit Protocol



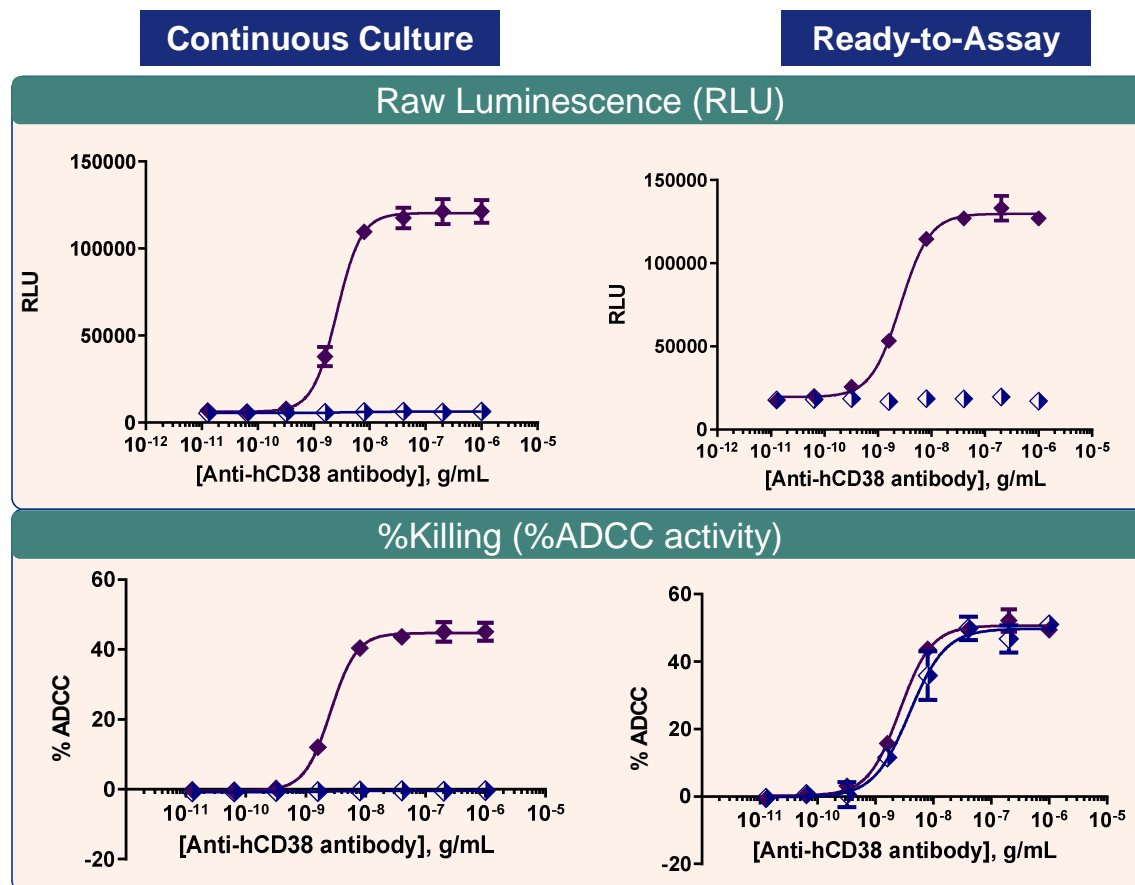
Bioassay Kit Includes Required Reagents for the Assay



*Effector cells sold separately

Continuous Culture vs Ready-to-Assay Cell Formats

KILR® Raji ADCC Bioassay Kit Performance



Condition	S/B	EC ₅₀ , ng/mL
Continuous Culture	19	2.6
Assay-ready	6.7	2.6

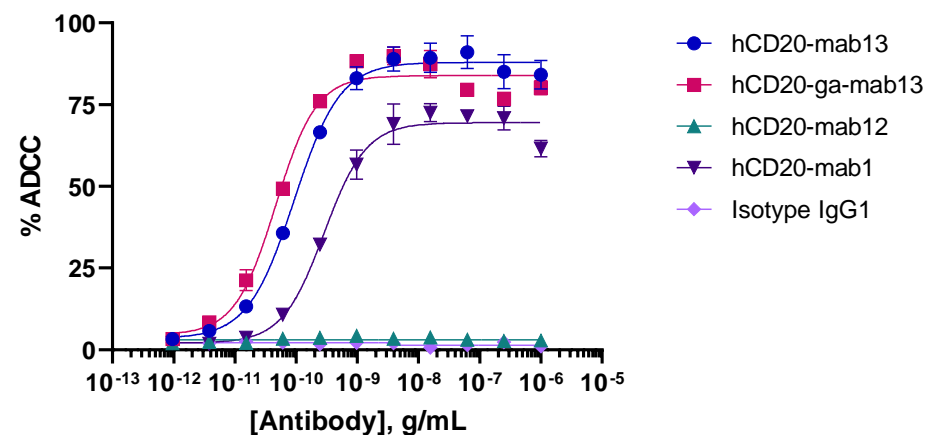
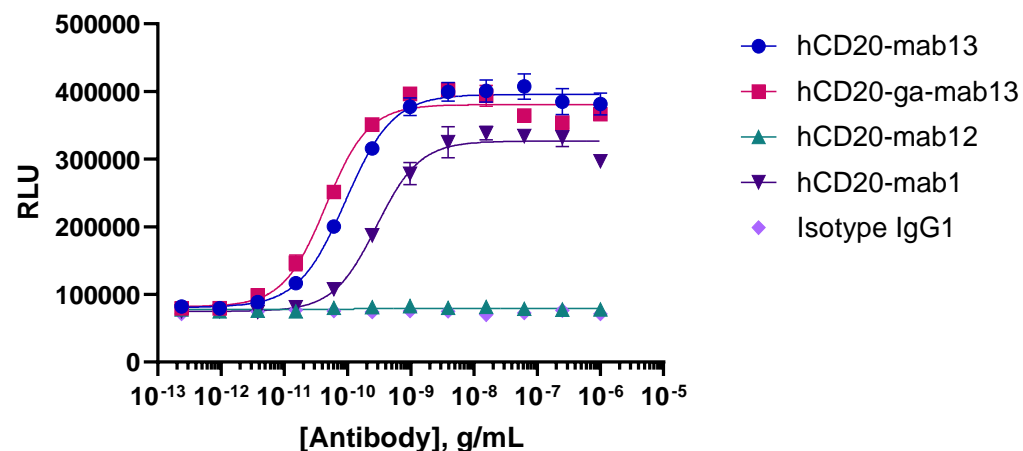
Excellent Concordance between EC₅₀

Condition	E _{Max}	EC ₅₀ , ng/mL
Continuous Culture	45%	2.6
Assay-ready	51%	2.6

Comparable % Killing (% ADCC) with KILR Raji Bioassay Cells Relative to Continuous Culture

Suitable for Screening and Rank Ordering of Antibodies

KILR® Raji ADCC Bioassay Kit



Sample	Description	S/B	HillSlope	E _{Max}	EC ₅₀ , pg/mL
hCD20-mab13	Non-fucosylated anti-CD20 (Rituximab)	5.4	1.152	88%	92.8
hCD20-ga-mab13	Non-fucosylated anti-CD20 (Obinituzimab)	5.2	1.307	84%	46.5
hCD20-mab12	Fc Null variant of Rituximab	--	--	--	--
hCD20-mab1	Wild-type Rituximab	4.5	1.270	69%	288
Isotype (IgG1)	Human IgG1 isotype control	--	--	--	--

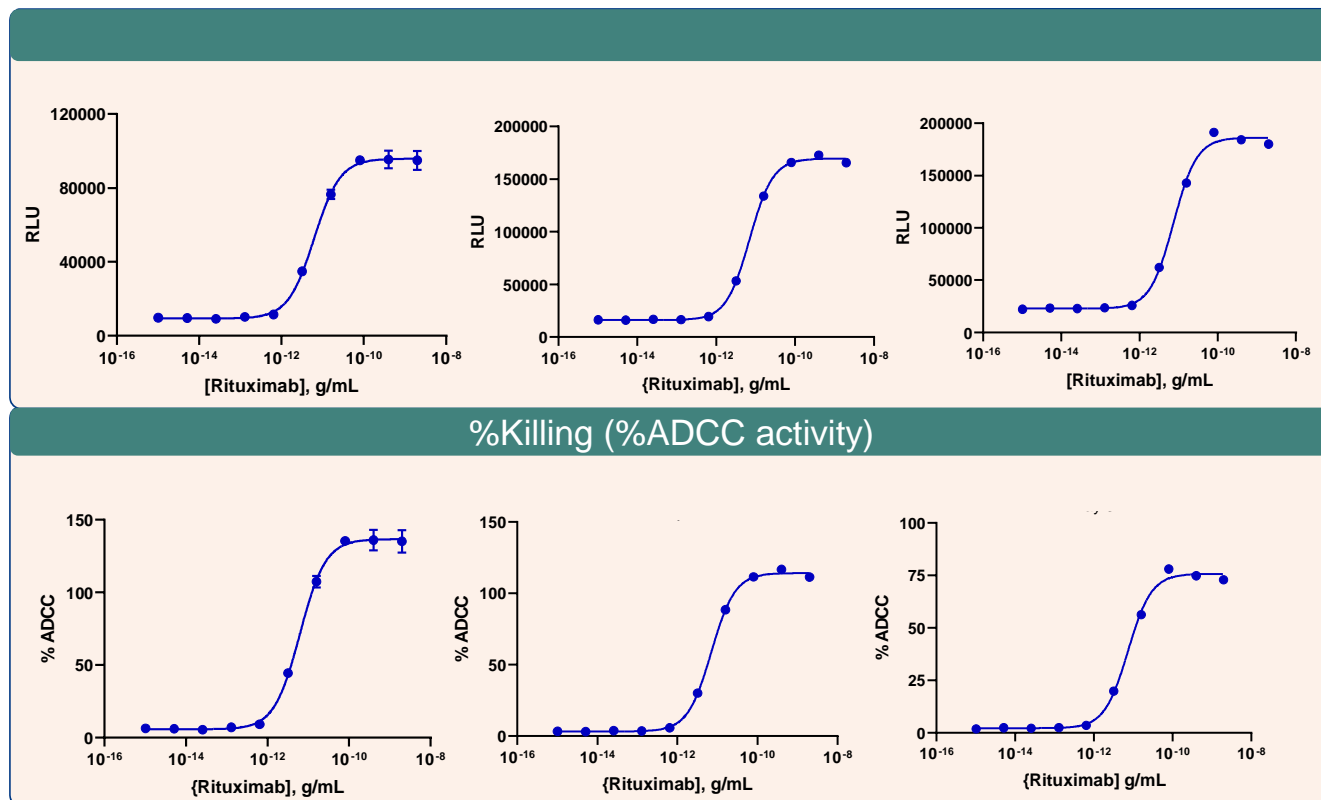
KILR Raji Bioassay allows discrimination of differences in Fab and effector regions of related antibodies

Repeatability (Over 3 Days); Single Analyst KILR® Raji ADCC Bioassay Kit

Day 1

Day 2

Day 3



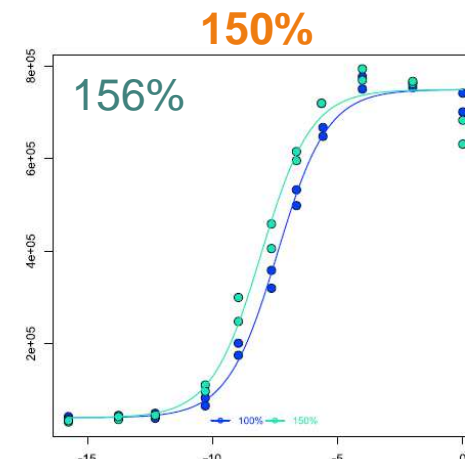
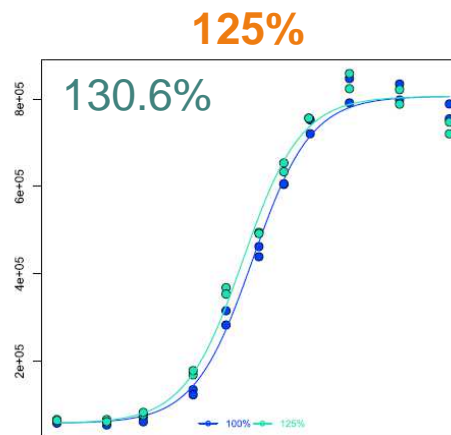
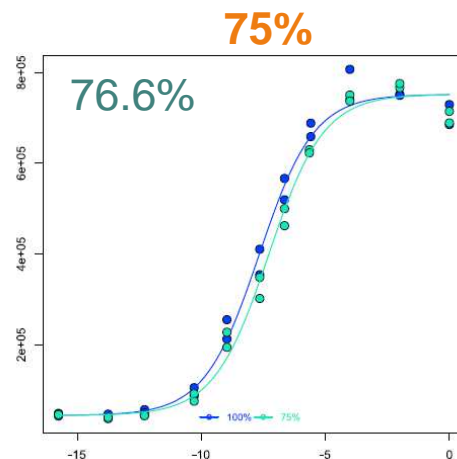
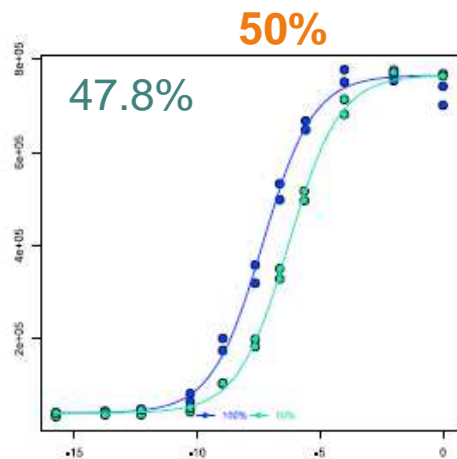
Parameter	Day 1	Day 2	Day 3	% RSD
EC ₅₀ , pg/mL	6.2	7.03	7.4	8.9%
S/B	9.8	10.6	8.3	12.2%
E _{Max} , %	135%	114%	75.8%	27.7%

Excellent inter-day repeatability

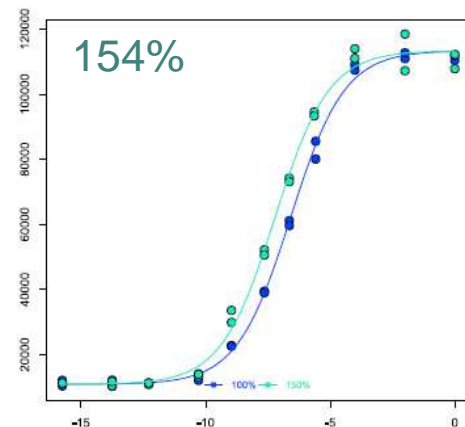
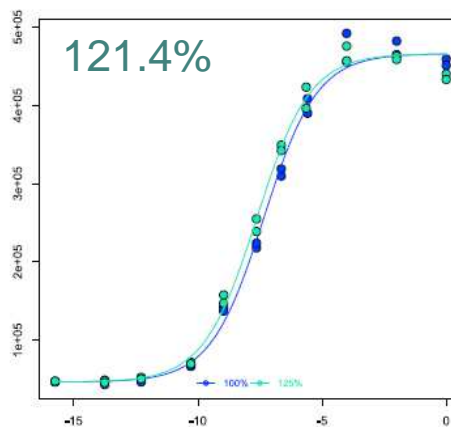
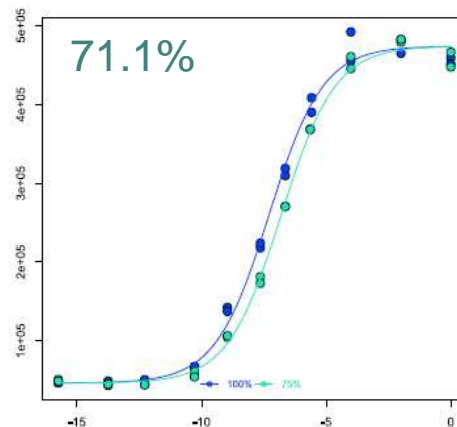
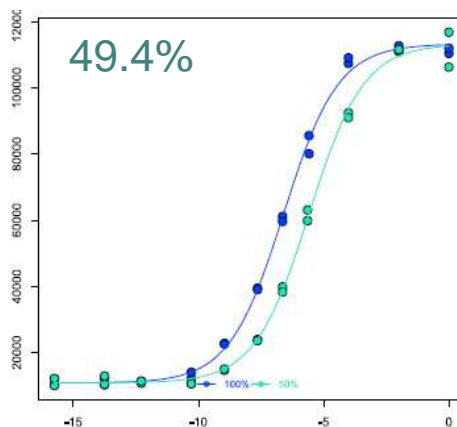
Consistent Performance Between Analysts (Relative Potency)

KILR® Raji ADCC Bioassay Kit

Analyst 1



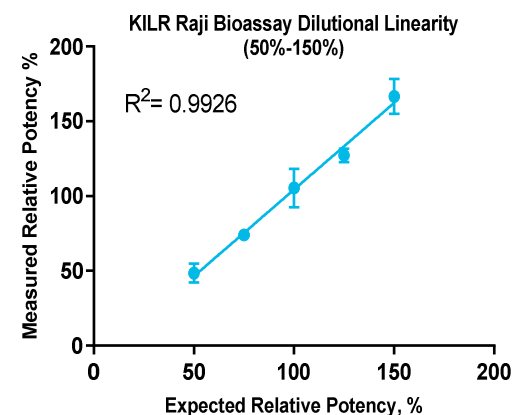
Analyst 2



Phase-Appropriate Qualifications

Suitability of KILR® Raji Bioassay Cells for Relative Potency Assays

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

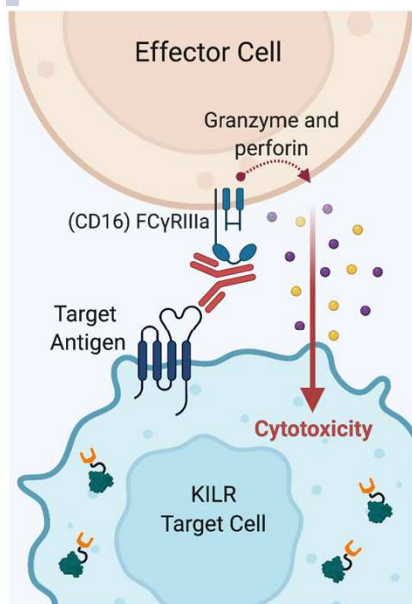
Assay demonstrates very good accuracy, repeatability, intermediate precision, and dilutional linearity

Multiple MOAs Supported by KILR[®] Cytotoxicity Assays

ADCC

Antibody-Dependent Cellular Cytotoxicity

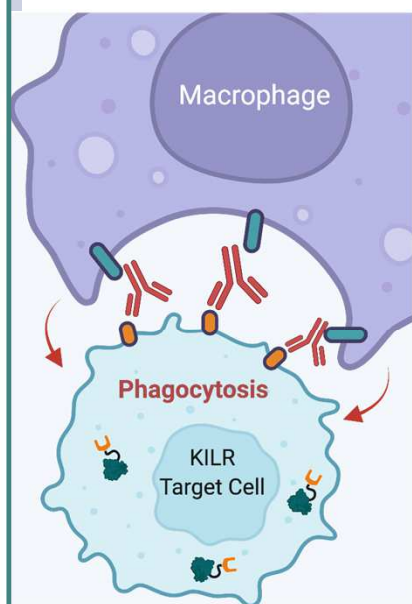
Effector cells engage with the target cells through the antibody and kill



ADCP

Antibody-Dependent Cellular Phagocytosis

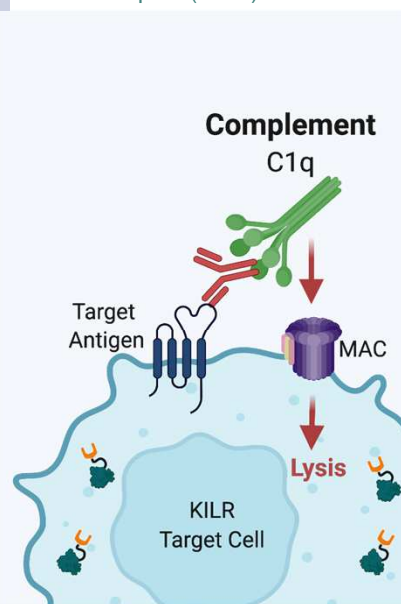
Fc-dependent phagocytosis and lysosomal degradation and kill



CDC

Complement Dependent Cytotoxicity

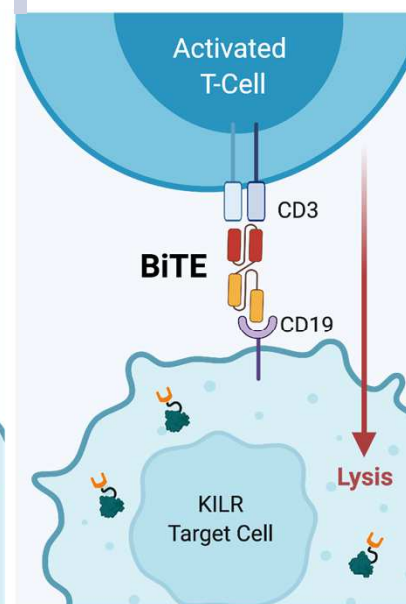
Activated complement system leads to formation of membrane attack complex (MAC)



TCR

T-cell Redirection (with Bi-Specific Ab)

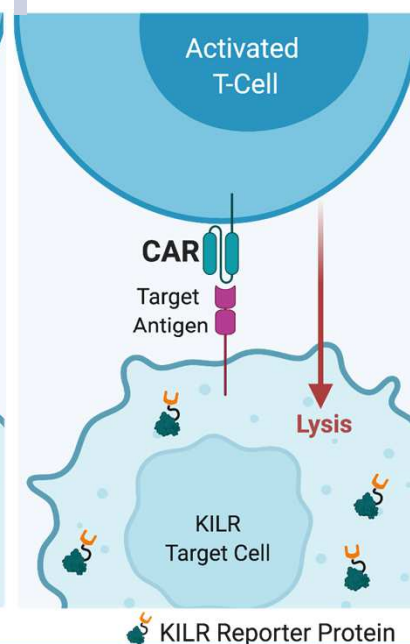
Bi-specific antibody engages T-cell with cancer cells for killing



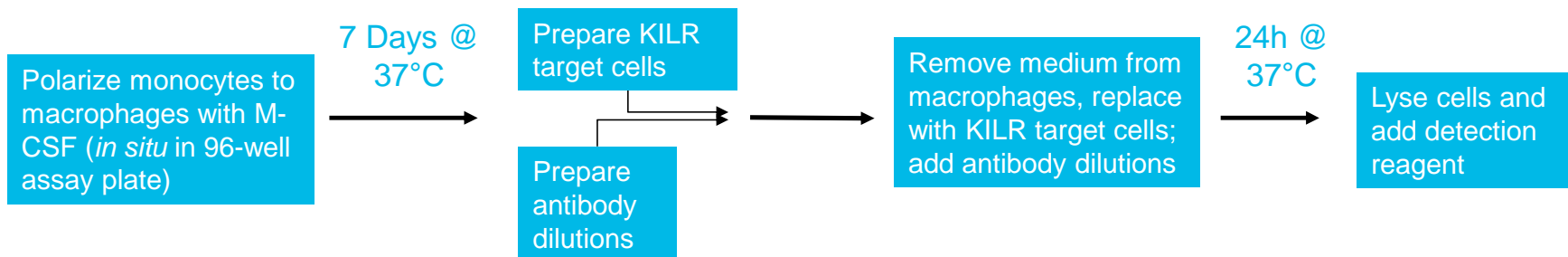
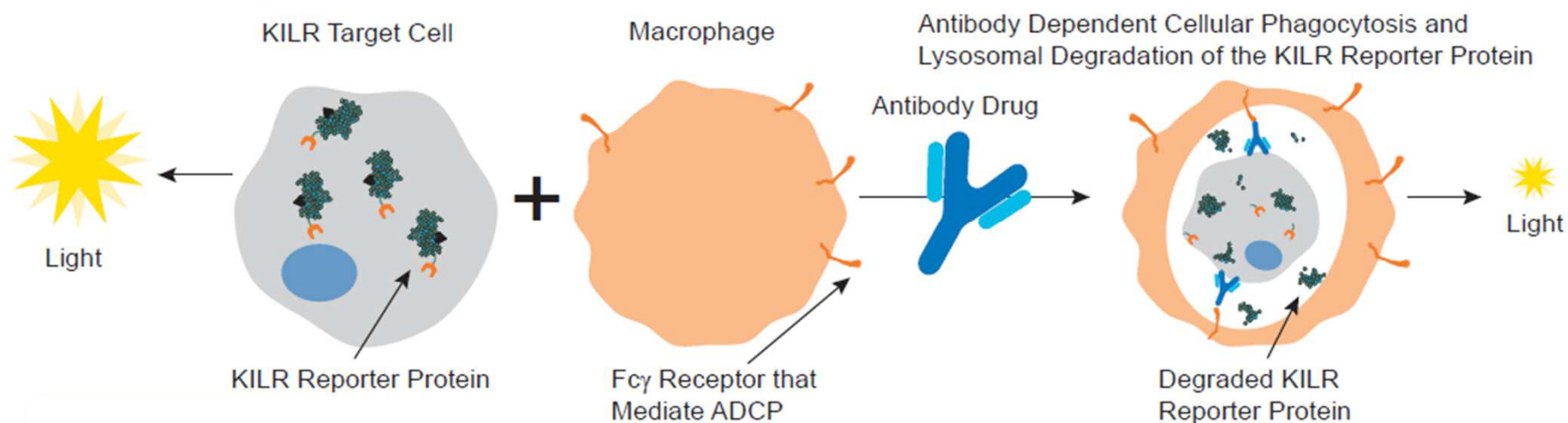
CAR-T

Chimeric antigen receptor (on) T-cells

Engineered T-cells recognize and kill cancer cells

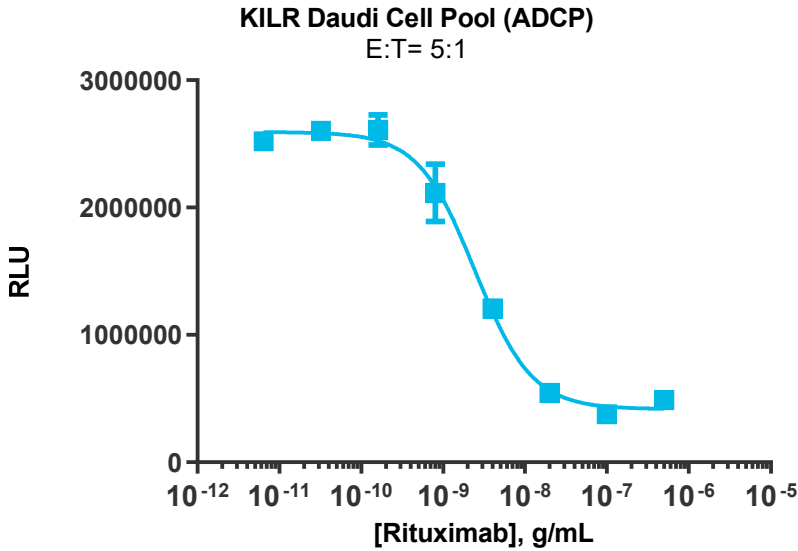


KILR ADCP Assay Concept and Workflow

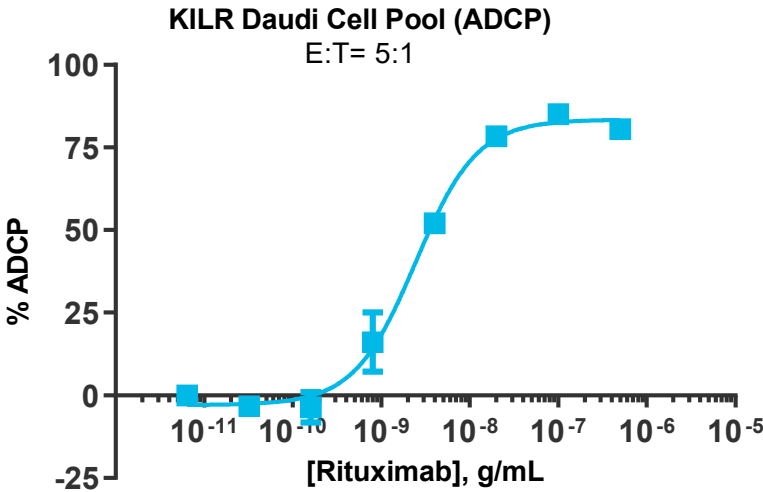


Measuring ADCP in KILR[®] Daudi Cells

Raw Data



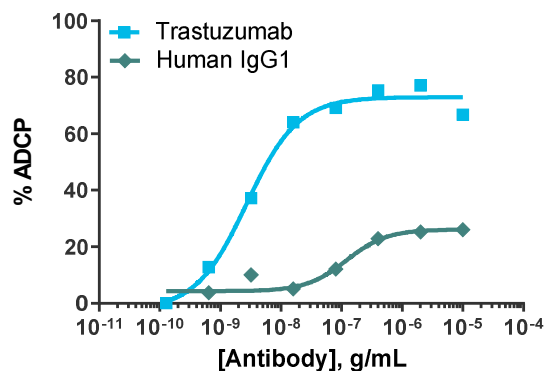
% Killing (% ADCP)



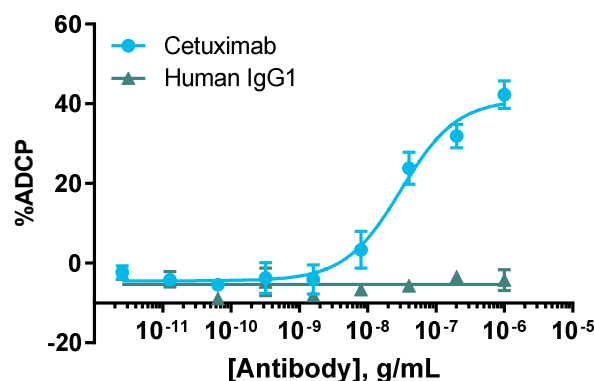
Sample	S/B	E _{Max}	EC ₅₀ , ng/mL
Rituximab	6.2	83%	2.44

ADCP Observed for Diverse Antigens in Solid and Heme Cancer Models

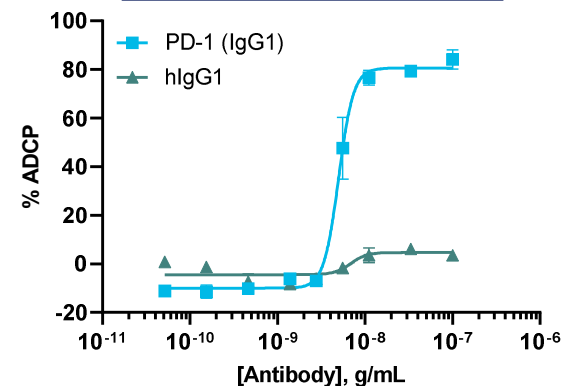
HER2+ Breast Cancer



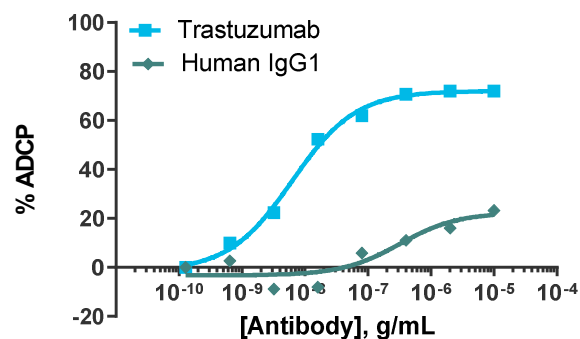
HER2- Breast Cancer



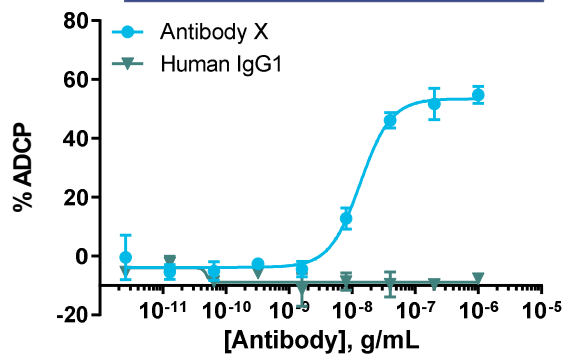
PD-1+ T Cell Model



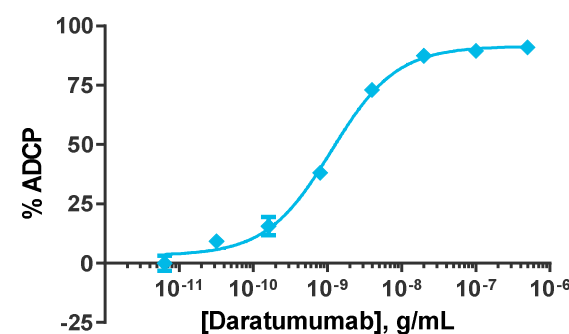
HER2+ Gastric Cancer



HER2- Breast Cancer



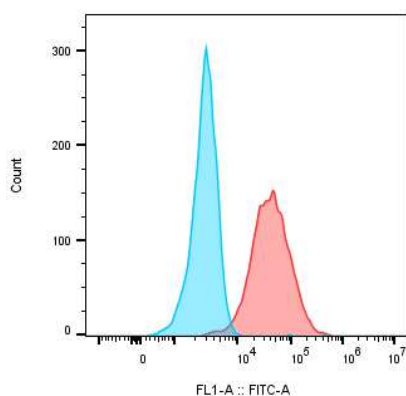
CD38+ B Cell Model



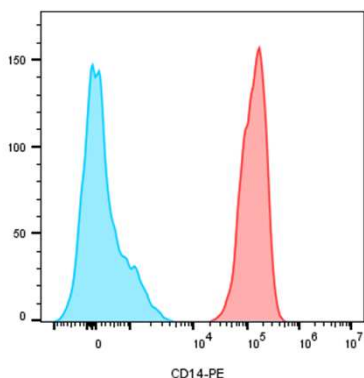
Higher Percentage of Phagocytosis Seen with Plate-Based, KILR[®] Assay Compared to Flow-Based Assay Formats

M0 Macrophage Markers

CD11b

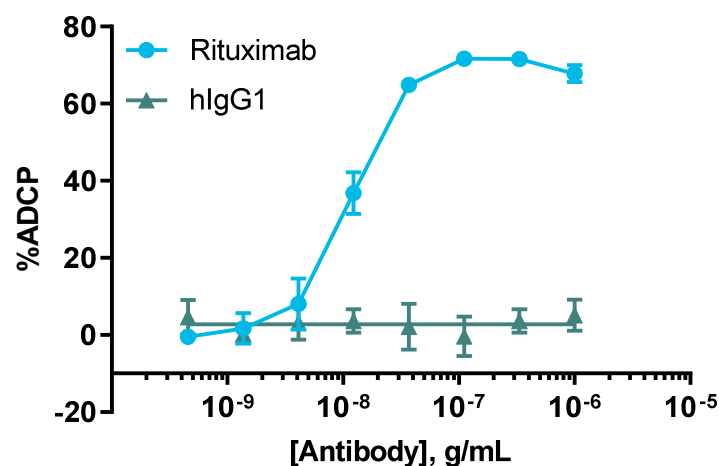


CD14

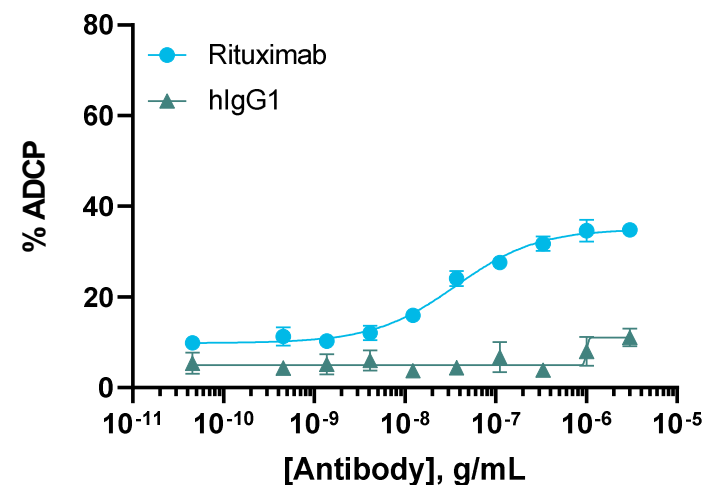


ADCP Results

EFC (KILR)



Flow Cytometry



ADCP Method	Target Cells	E:T	S/B	EMax, %	EC50, ng/mL
EFC (KILR)	KILR Raji	5:1	3.6	71.6%	11.4
Flow Cytometry	CFSE-labeled Raji	5:1	3.5	35%	36.3

Evaluate ADCP Activity of Therapeutics with IgG1 and IgG4(S228P) Fc Regions

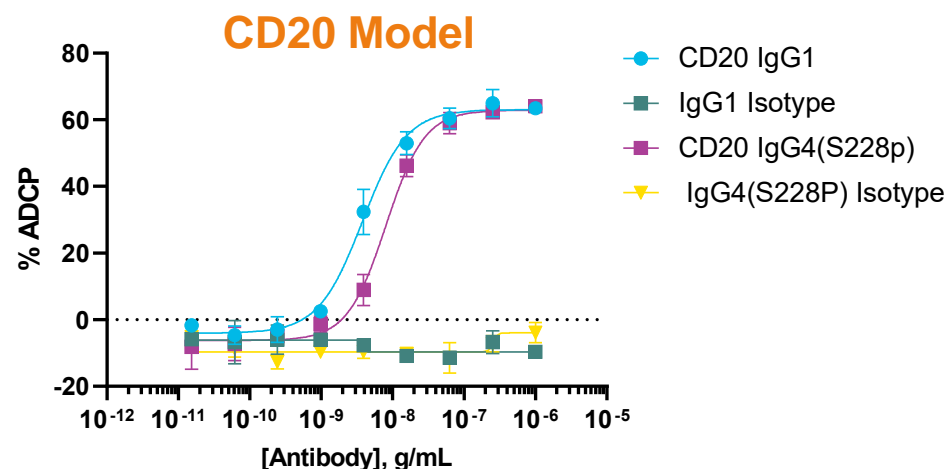
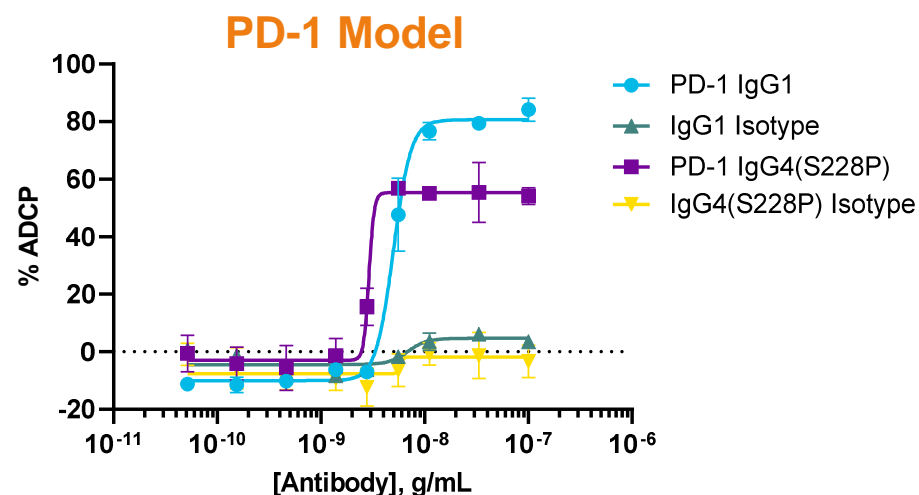
IgGx Affinity for Fc Receptors (impact on immune-mediated killing)

ADCC: IgG1 \geq IgG3 > IgG2 \geq IgG4

CDC: IgG3 > IgG1 > IgG2 > IgG4

ADCP: IgG1 \geq IgG3 > IgG2 > IgG4

IgG4(S228P): A specific variant of IgG4, commonly used for anti-PD-1 therapeutics, mediates ADCP



Results indicate developers using IgG4(S228P) Fc region need to evaluate ADCP activity of their therapeutic

Summary and Conclusions

KILR[®] cytotoxicity platform offers MOA-based assays for variety of cell-mediated cytotoxicity applications such as:

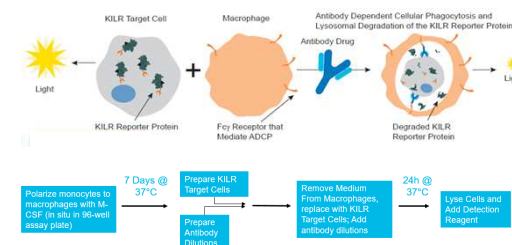
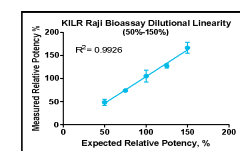
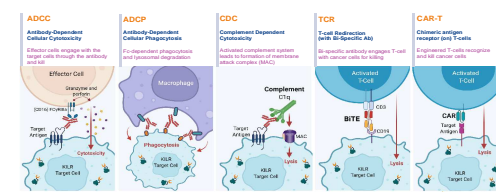
ADCC, ADCP, CDC, T-cell Redirection and CAR-T

KILR ADCC Bioassays allows discrimination of differences in Fab and Fc regions to enable rank ordering of antibodies

KILR ADCC Bioassays, when used in combination with KILR CD16 Effector Cells, produce highly accurate and precise data suitable for use in relative potency assays

KILR ADCP Bioassays provide a robust, MOA-reflective readout for ADCP in a simple, plate-based assay format that minimizes macrophage handling

KILR ADCP Bioassays have utility for evaluating ADCP activity of therapeutics with IgG1 and IgG4(S228P) Fc regions



Visit discoverx.com/kilr-bioassays to learn more