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

Andrew Green
Sr. Business Development Manager
Eurofins DiscoverX

OUR EXPERTISE IN YOUR HANDS.
DISCOVER CONFIDENTLY.

Eurofins DiscoverX Is a Global Leader in Cell-Based Assays for Screening, Profiling, Potency & Lot Release Programs

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From Discovery to Development to Clinic to Post-Market

20+ Years of Enabling Drug Discovery and Development Programs

San Francisco Bay Area, California

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+
Qualified & MOA-based bioassays

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

3 Certified CRO Partners
Scientific training to enable global CROs

ICH-Based Bioassay Qualification
Facilitate downstream validation studies

Dedicated Scientific Support
Experienced team providing scientific support

20+ Successful Assay Transfers
At clients/affiliated CRO sites

70+ Global Programs
For potency, stability and NAb testing

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

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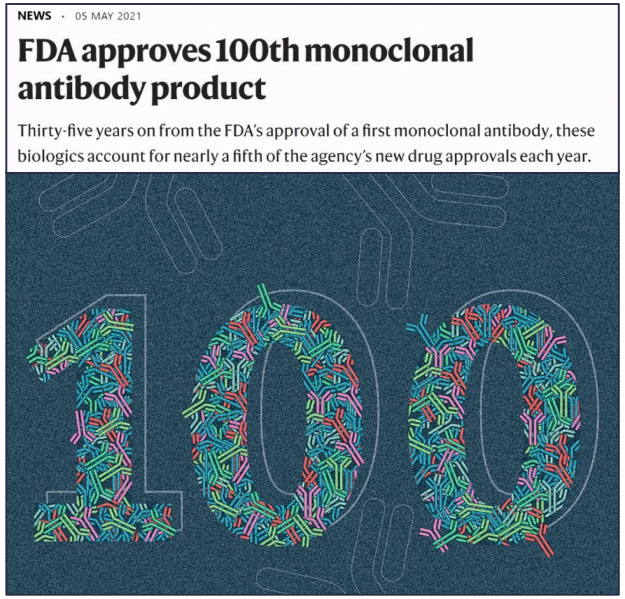
Therapeutic mAbs are the fastest growing class of biological therapeutics, and over 100 mAbs are already approved by the FDA

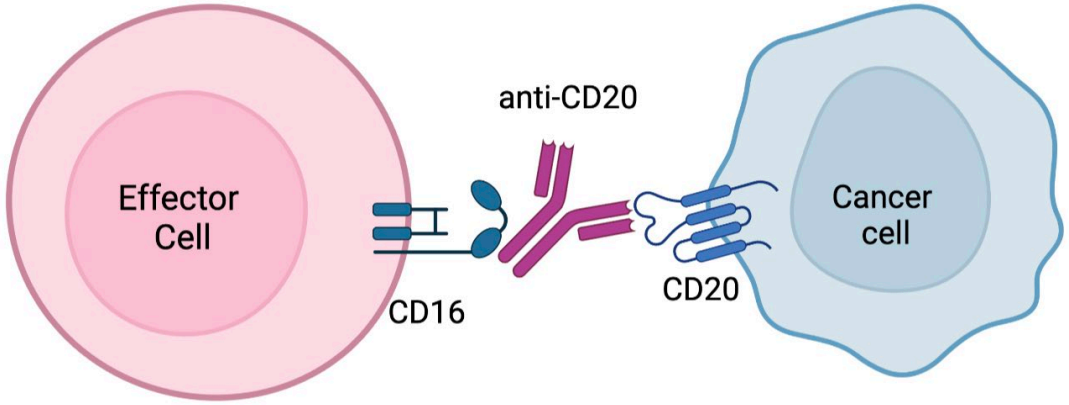
Many of these therapeutic antibodies work by activating antibody-dependent cell-mediated cytotoxicity (ADCC) or other effector-mediated functions (ADCP, CDC, T-cell Redirection)

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


NEWS · 05 MAY 2021

FDA approves 100th monoclonal antibody product

Thirty-five years on from the FDA's approval of a first monoclonal antibody, these biologics account for nearly a fifth of the agency's new drug approvals each year.



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

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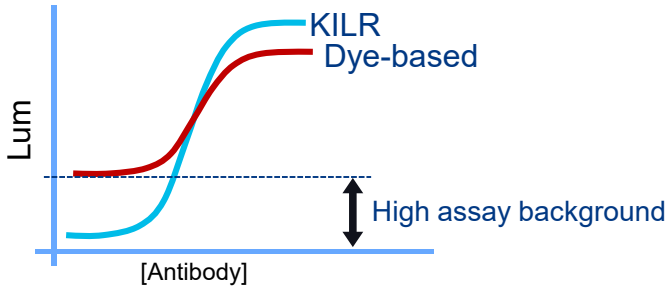
What scientists face….

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

- KILR® assays are non-radioactive and not leaky

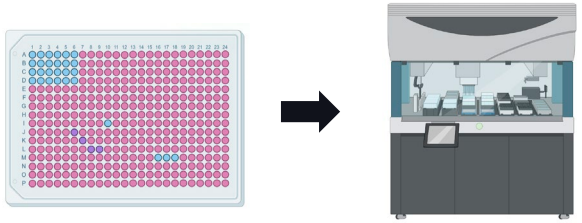
Little to no background



Low assay throughput restricts screening and characterization efficiency

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

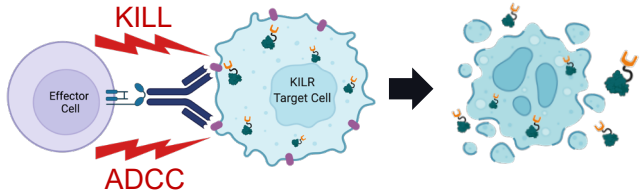
Ease of implementation from screening to lot release



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

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

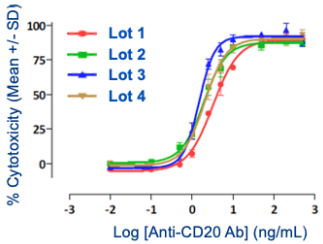
Physiologically-reflective of TRUE MOA



High donor-to-donor variability associated with effector cells

- KILR CD16 Effector Cells are single donor-derived

Eliminate donor variability



Enzyme Fragment Complementation (EFC) Technology

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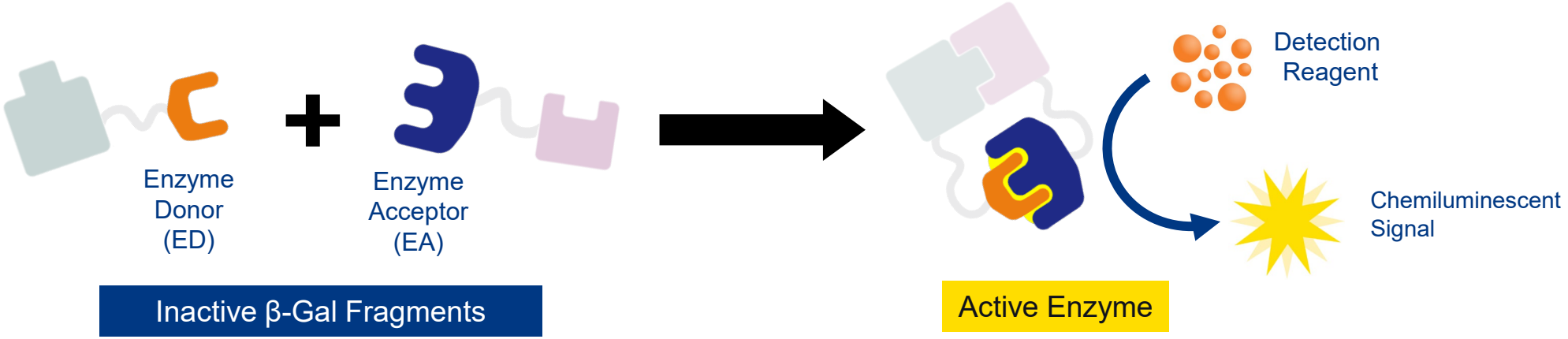
Enabling Technologies with a Flexible Platform based on a Split β-Galactosidase Enzyme



Homogenous

- Simple add-and-read assay format
- No media change, shaking or filtration

Sensitive Detection

- Enzymatically-amplified Assay
- High precision & accuracy

Robust

- Large dynamic range
- High assay reproducibility

Luminescence Readout

- Easily quantified luminescence read-out
- Compatible with most readers

Easy to Transfer

- Assay protocol is similar across the platform
- Detailed user manuals

KILR® Cytotoxicity Assay Overview
ADCC Example

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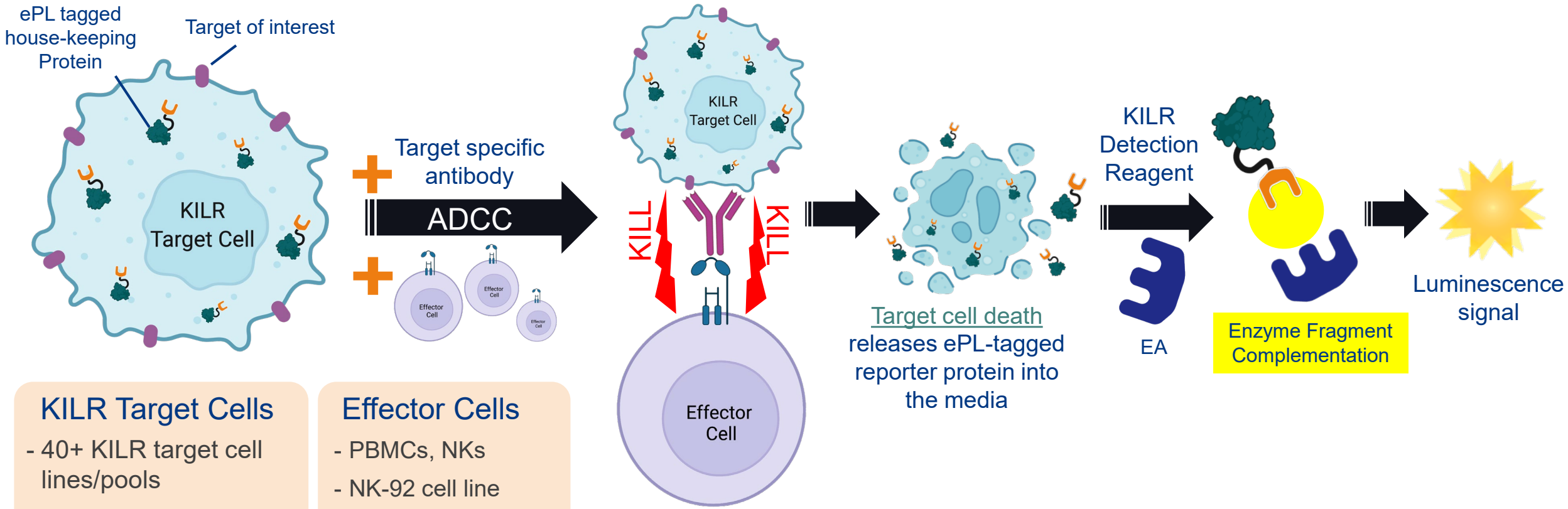
An easy-to-use assay to specifically measure target cell death



KILR Target Cells

- 40+ KILR target cell lines/pools
- 3 KILR RTU models
- Target cells generated using KILR retroparticles (DIY)

Effector Cells

- PBMCs, NKs
- NK-92 cell line
- CAR-T
- KILR CD16 Effectors Cells

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

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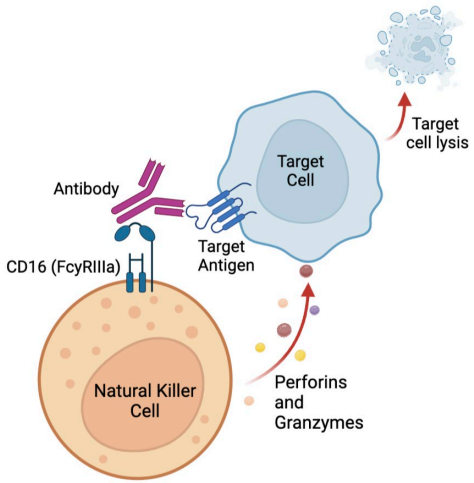
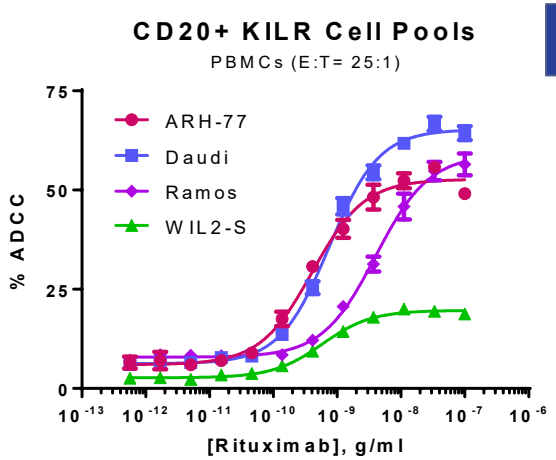
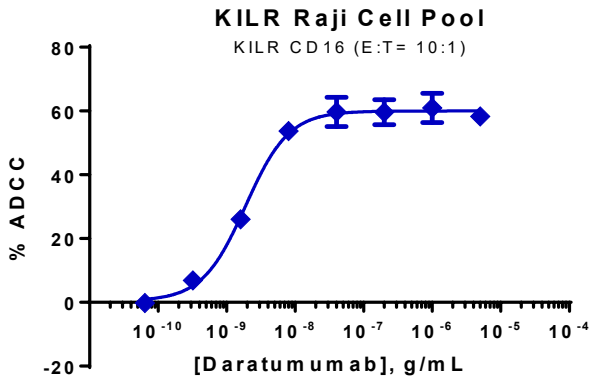


CD20

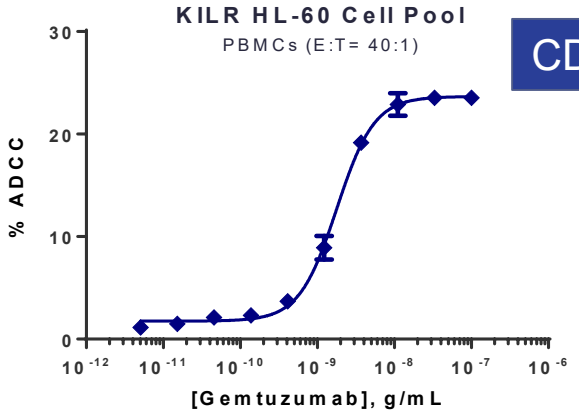


CD38



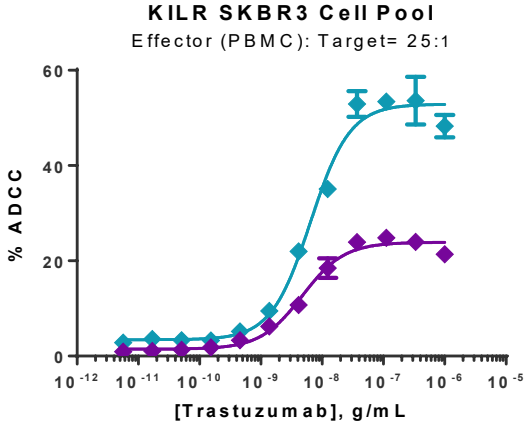
Sample	E_{Max}	EC_{50}, ng/mL
Daratumumab	60%	1.89

CD33



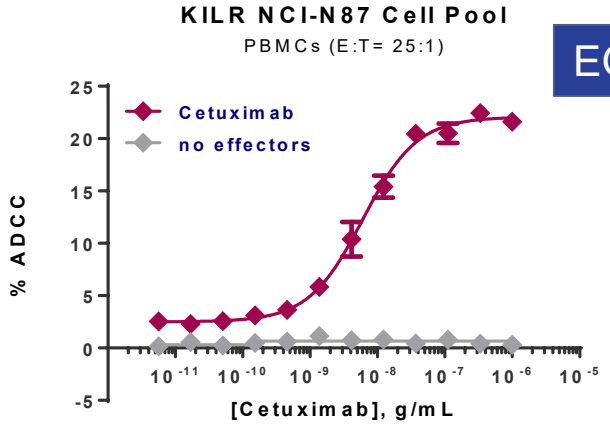
Sample	E_{Max}	EC_{50}, ng/mL
Gemtuzumab	24%	1.78

HER2



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

EGFR



Sample	E_{Max}	EC_{50}, ng/mL
Cetuximab	22%	6.04

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

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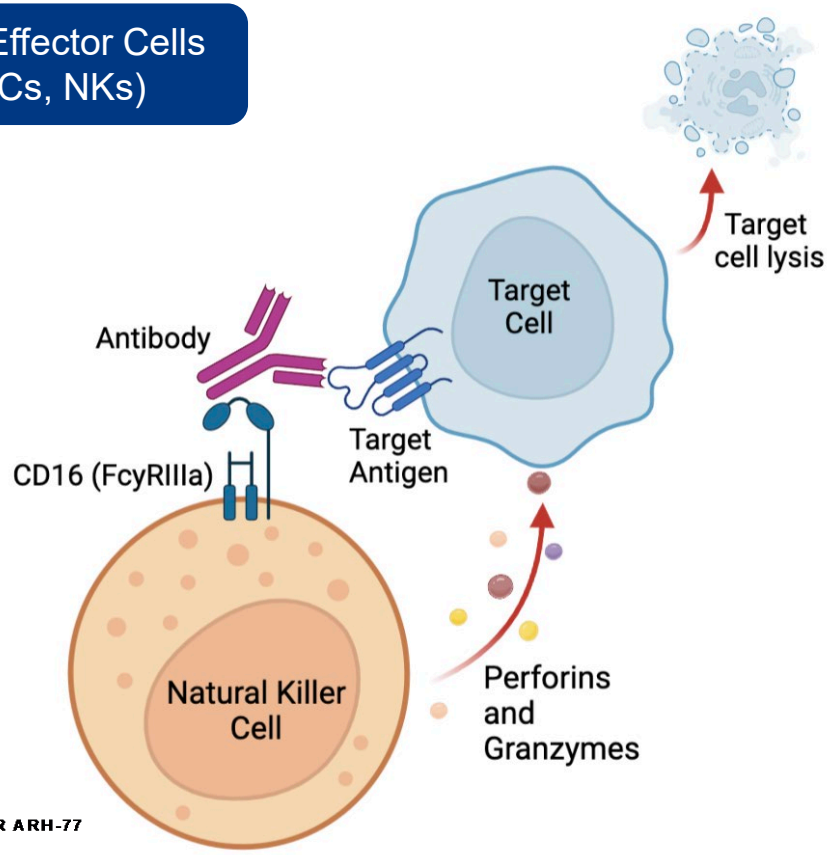
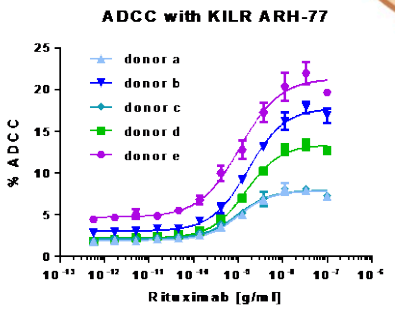
Isolated Effector Cells (PBMCs, NKs)

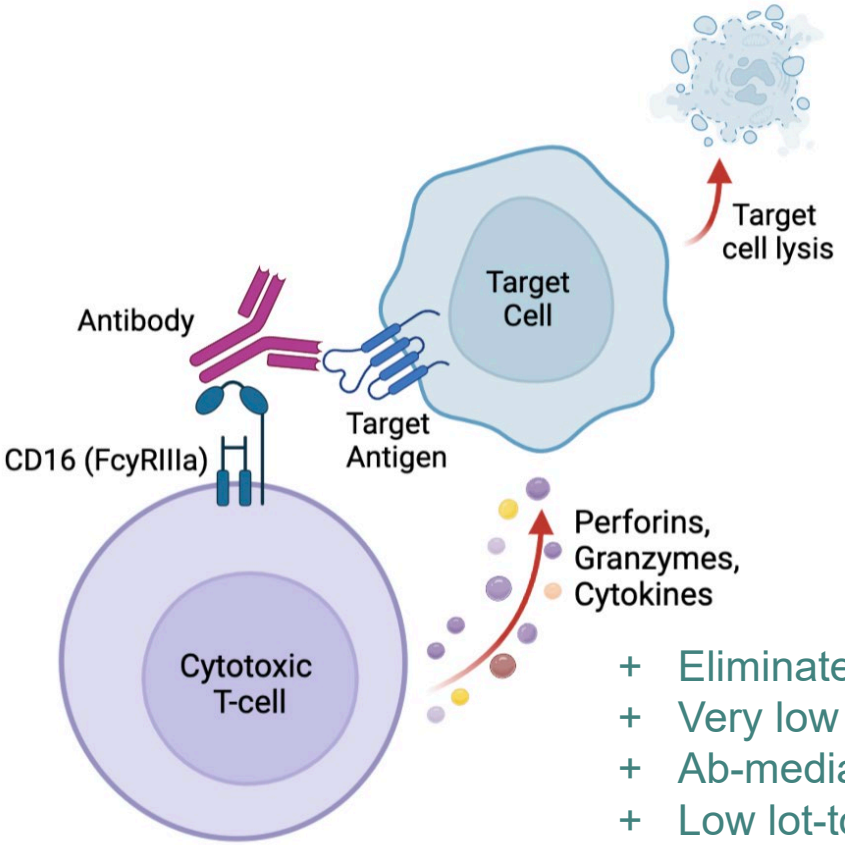


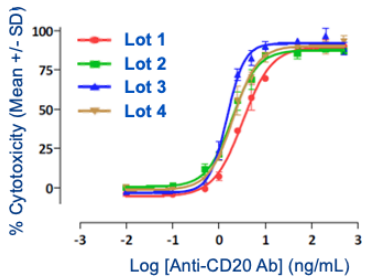


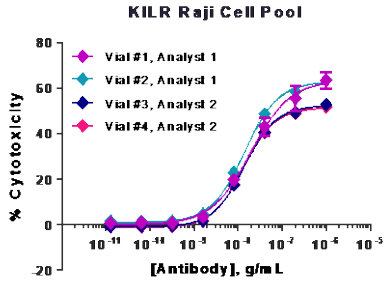
- High donor variability
- Heterogenous population (PBMCs)
- High lot-to-lot variability
- High background killing (NK cells)

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

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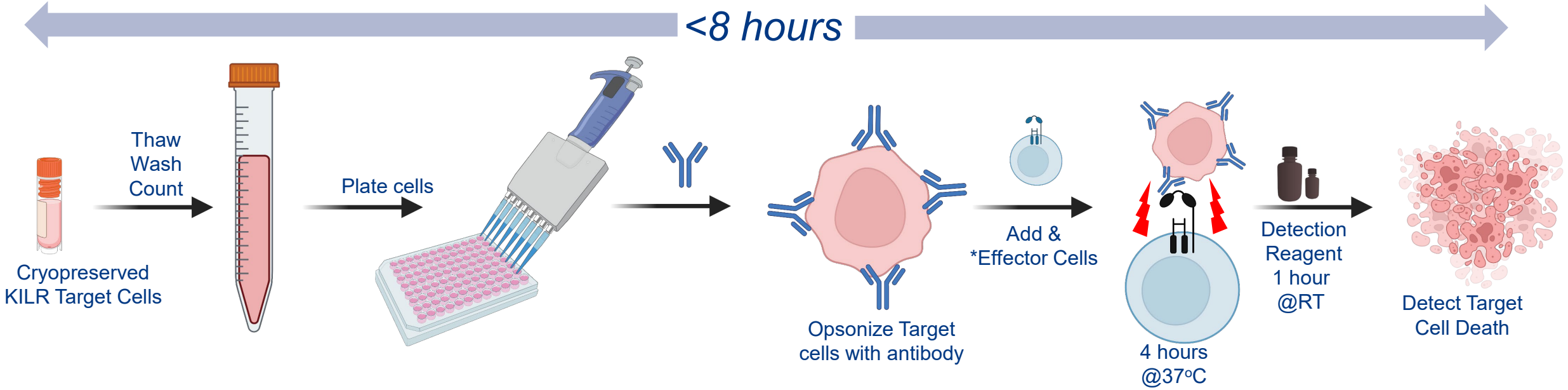
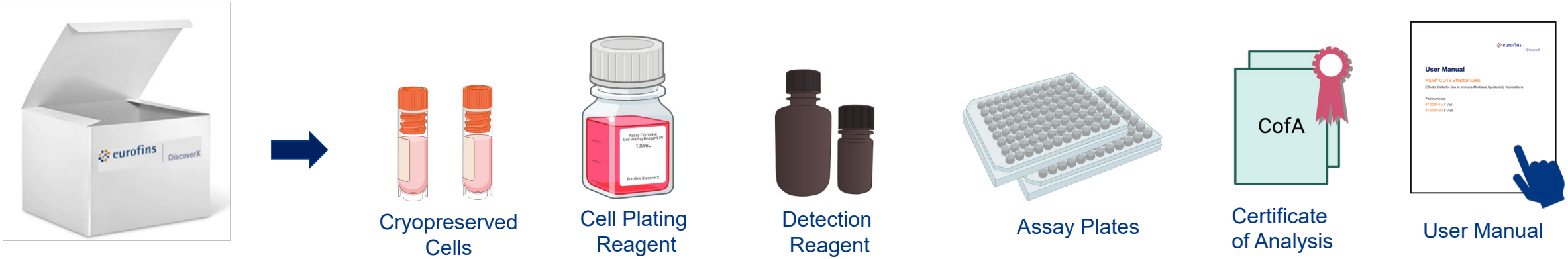


Bioassay Kit Includes Required Reagents for the Assay



*Effector cells sold separately

Continuous Culture vs Ready-to-Assay Cell Formats

KILR® Raji ADCC Bioassay Kit Performance

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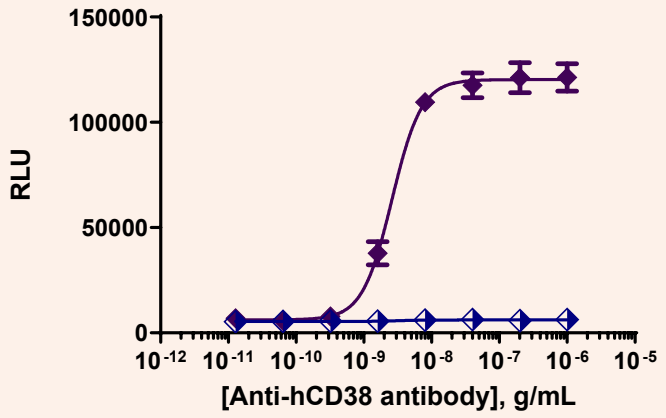
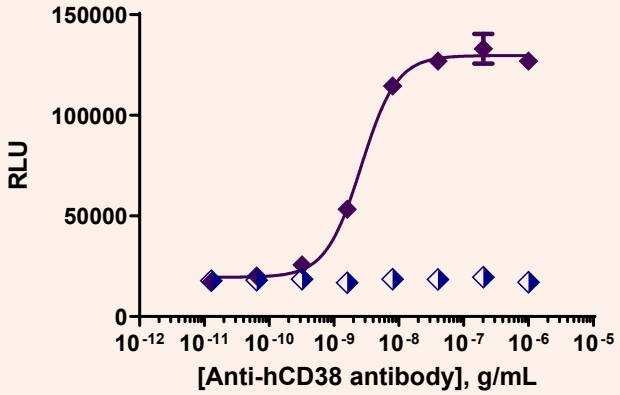
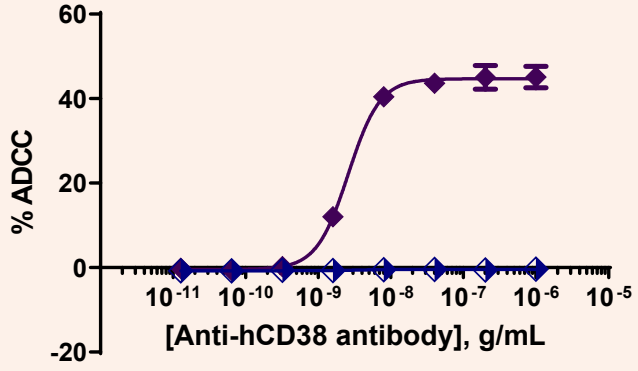
Continuous Culture | **Ready-to-Assay**

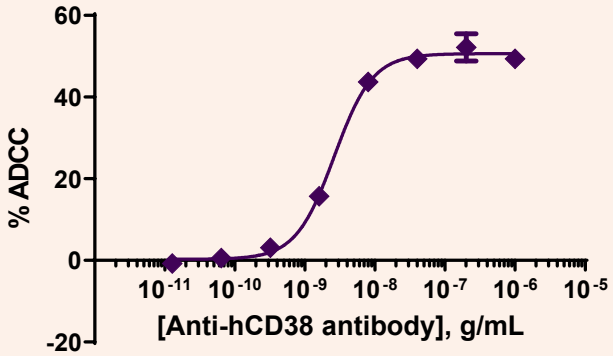
Raw Luminescence (RLU)





%Killing (%ADCC activity)





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

Excellent Concordance between EC_{50}

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

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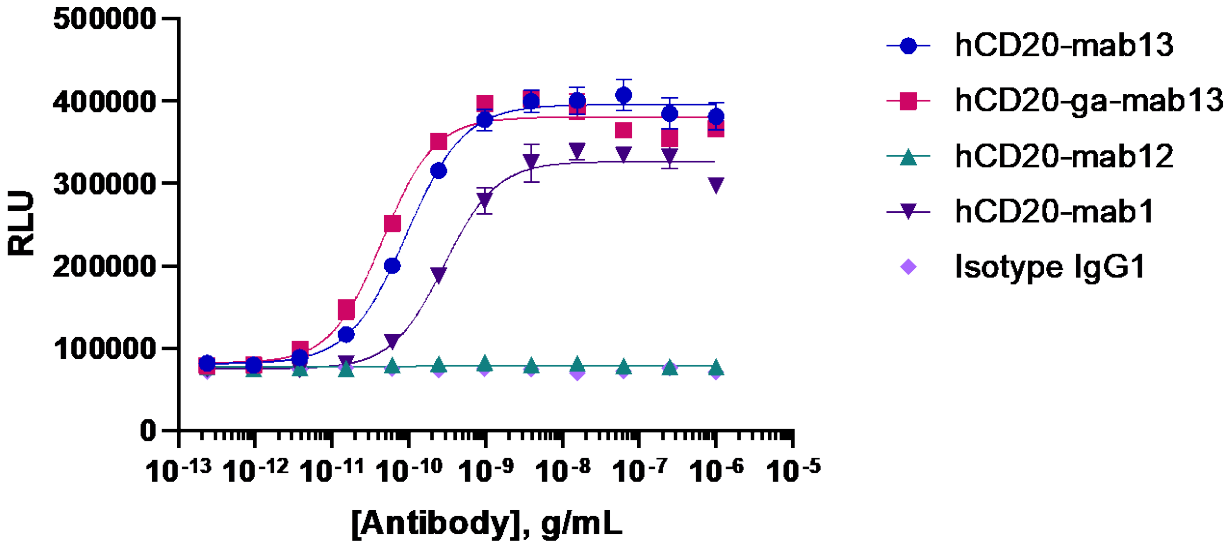

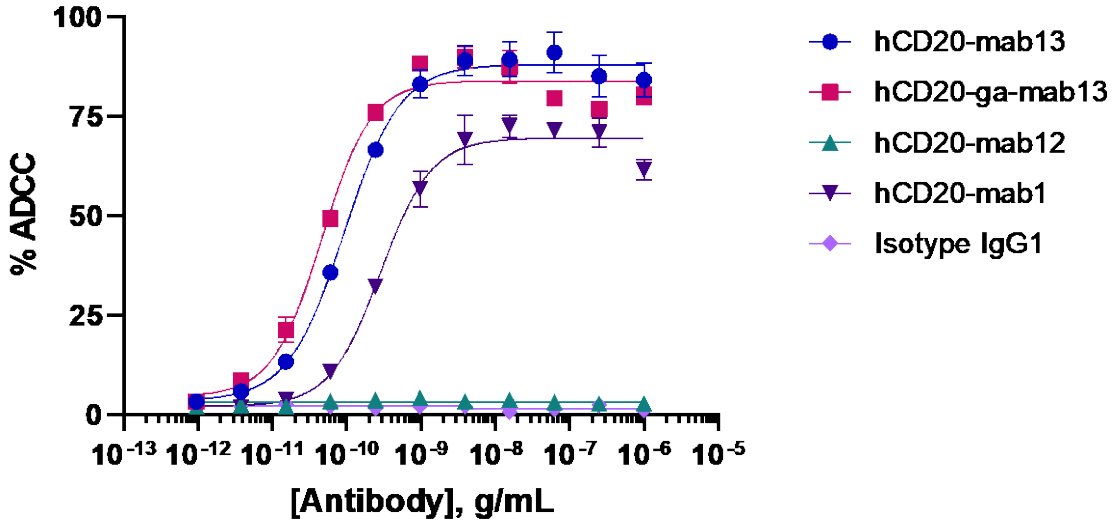

	Sample	Description	S/B	HillSlope	E_{Max}	EC_{50}, pg/mL
●	hCD20-mab13	Non-fucosylated anti-CD20 (Rituximab)	5.4	1.152	88%	92.8
■	hCD20-ga-mab13	Non-fucosylated anti-CD20 (Obinituzimab)	5.2	1.307	84%	46.5
▲	hCD20-mab12	Fc Null variant of Rituximab	--	--	--	--
▼	hCD20-mab1	Wild-type Rituximab	4.5	1.270	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

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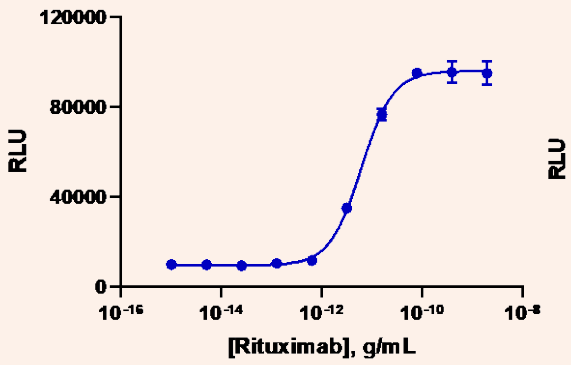
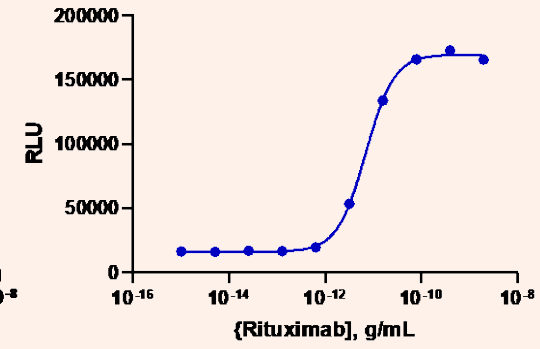
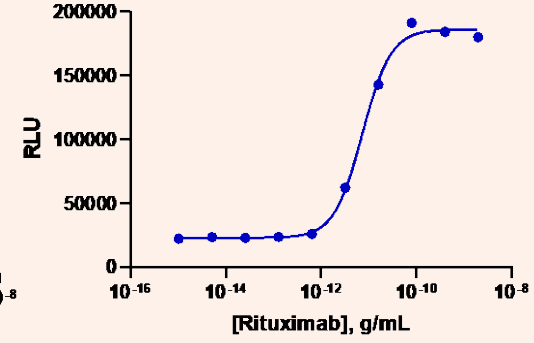
Day 1 **Day 2** **Day 3**

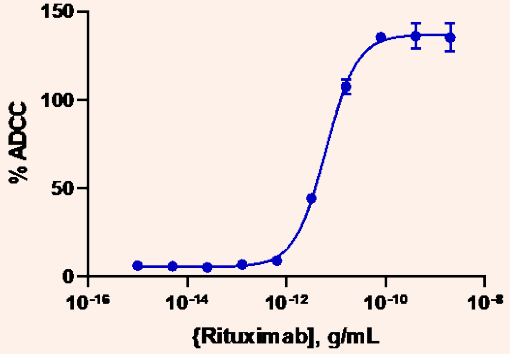
Raw Luminescence (RLU)

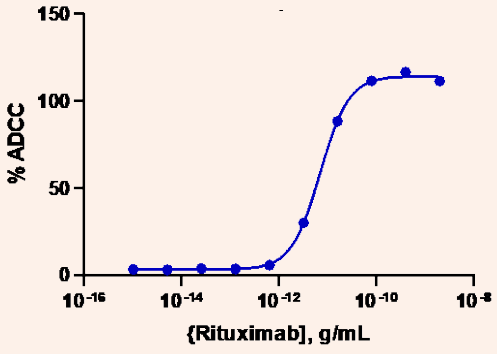


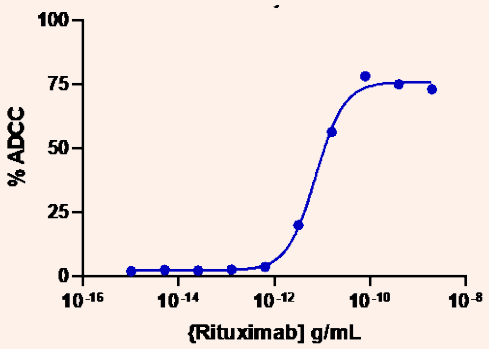




% Killing (% ADCC activity)







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 repeatabilty

Consistent Performance Between Analysts (Relative Potency)

KILR® Raji ADCC Bioassay Kit

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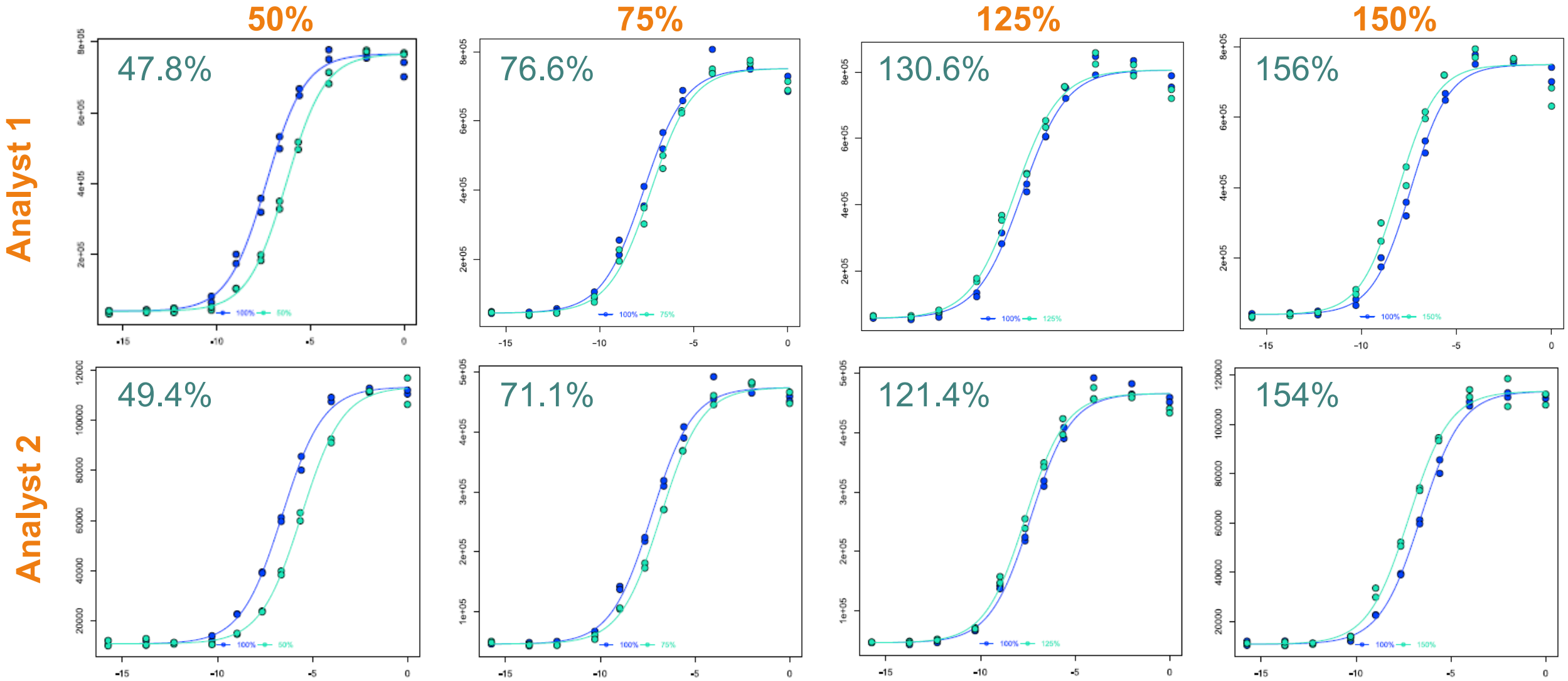

Phase-Appropriate Method Qualification

Suitability of KILR® Raji Bioassay Cells for Relative Potency Assays

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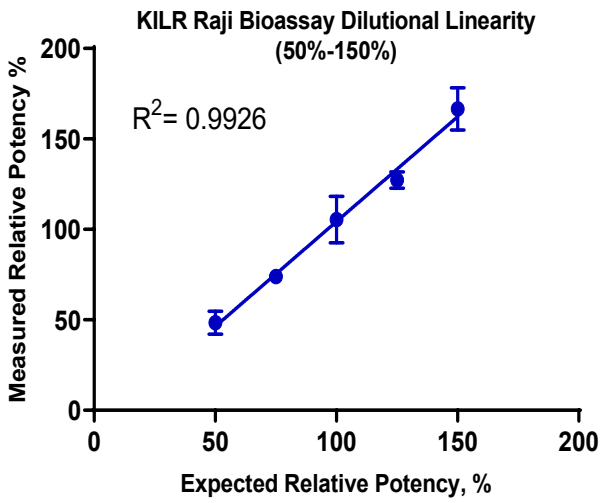
Nominal RP, %	Analyst	Observed RP, %	Average RP, %	% RSD	Average % Recovery
150	1	156	166.6	7.0	111.1%
	1	179.4			
	1	177.4			
	2	166			
	2	154.3			
`125	1	130.6	127.3	3.6	101.8%
	1	131.2			
	1	126			
	2	121.4			
100	1	82	106.5	13.0	106.5%
	1	116			
	1	112.7			
	1	98.4			
	1	117.8			
	2	112.2			
75	1	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

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

Multiple MOAs Supported by KILR® Cytotoxicity Assays

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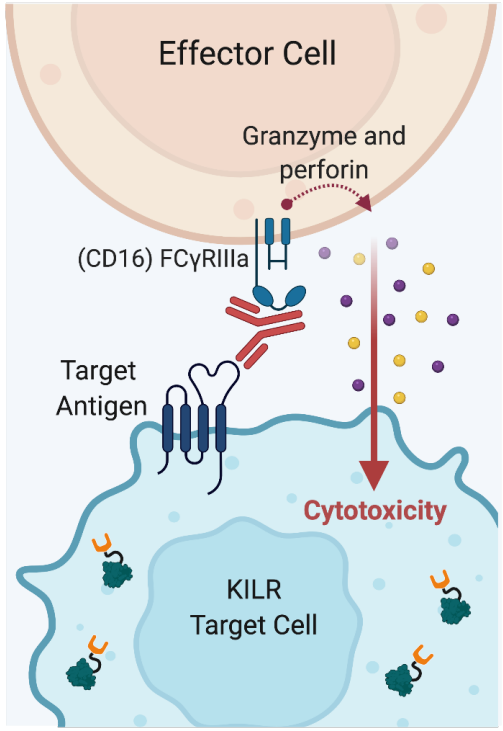
ADCC

Antibody-Dependent Cellular Cytotoxicity

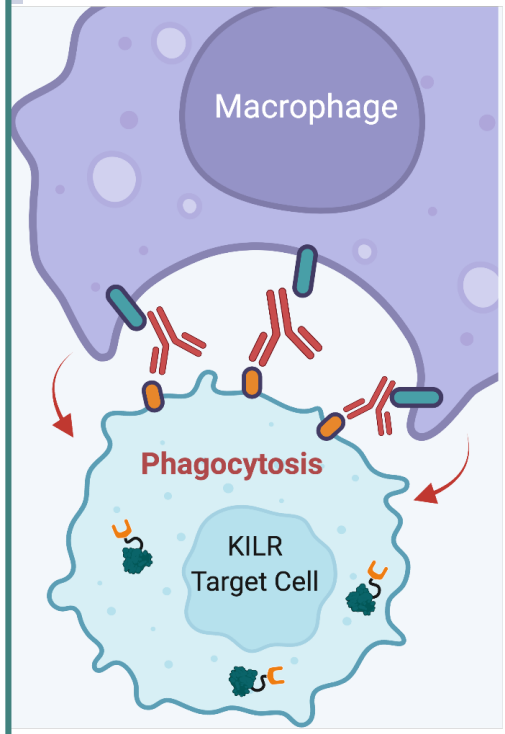
Effector cells engage with the target cells through the antibody and kill



ADCP

Antibody-Dependent Cellular Phagocytosis

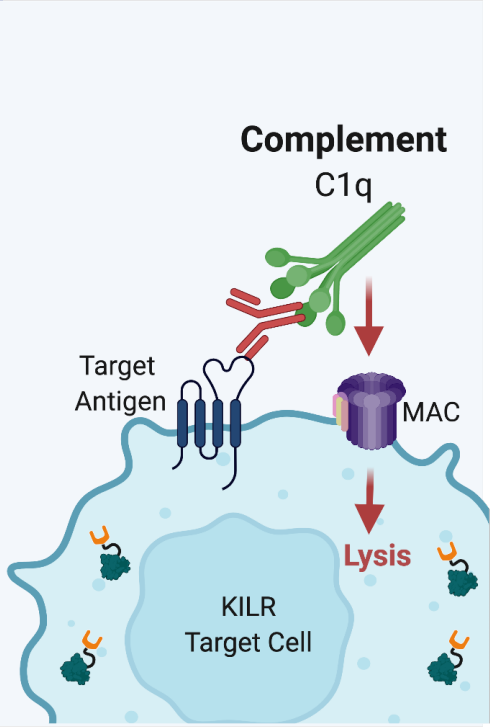
Fc-dependent phagocytosis and lysosomal degradation



CDC

Complement Dependent Cytotoxicity

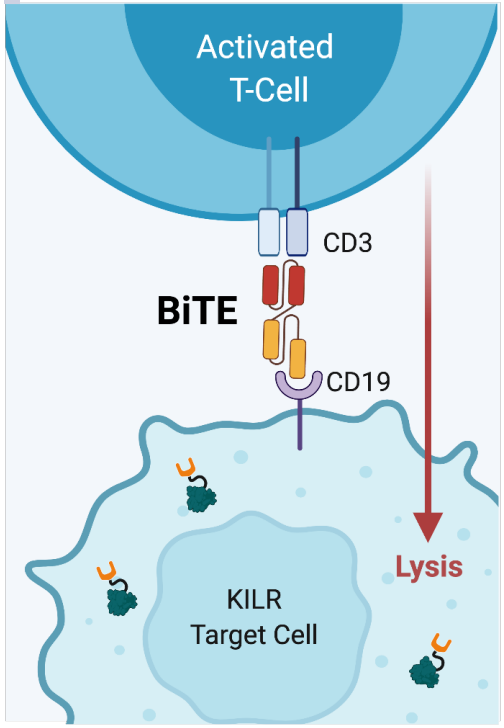
Activated complement system leads to formation of membrane attack complex (MAC)



TCR

T-cell Redirection (with Bi-Specific Ab)

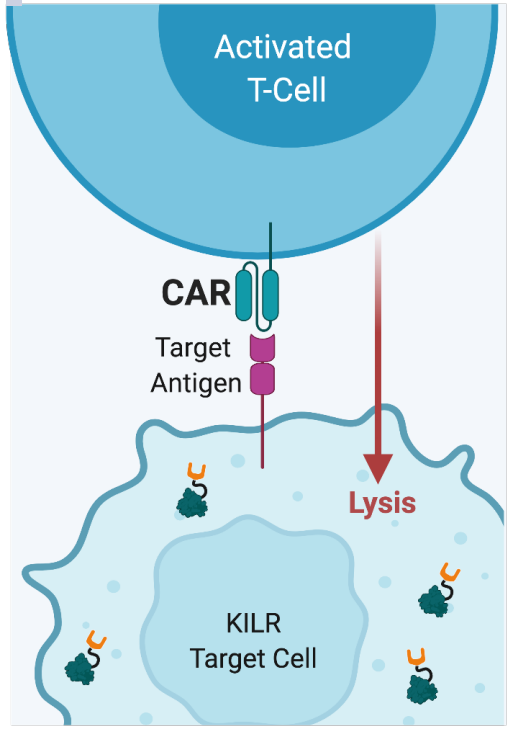
Bi-specific antibody engages T-cell with cancer cells for killing



CAR-T

Chimeric antigen receptor (on) T-cells

Engineered T-cells recognize and kill cancer cells



KILR Reporter Protein

KILR ADCP Assay Concept and Workflow

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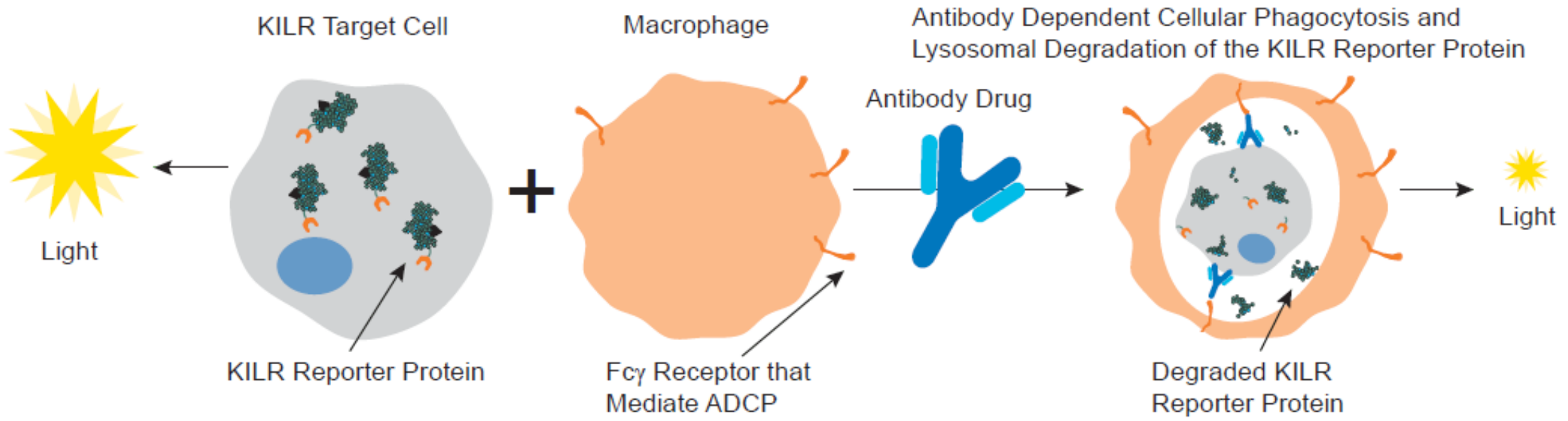

Polarize monocytes to macrophages with M-CSF (*in situ* in 96-well assay plate) → 7 Days @ 37°C → Prepare KILR target cells; Prepare antibody dilutions → Remove medium from macrophages, replace with KILR target cells; add antibody dilutions → 24h @ 37°C → Lyse cells and add detection reagent

Measuring ADCP in KILR® Daudi Cells

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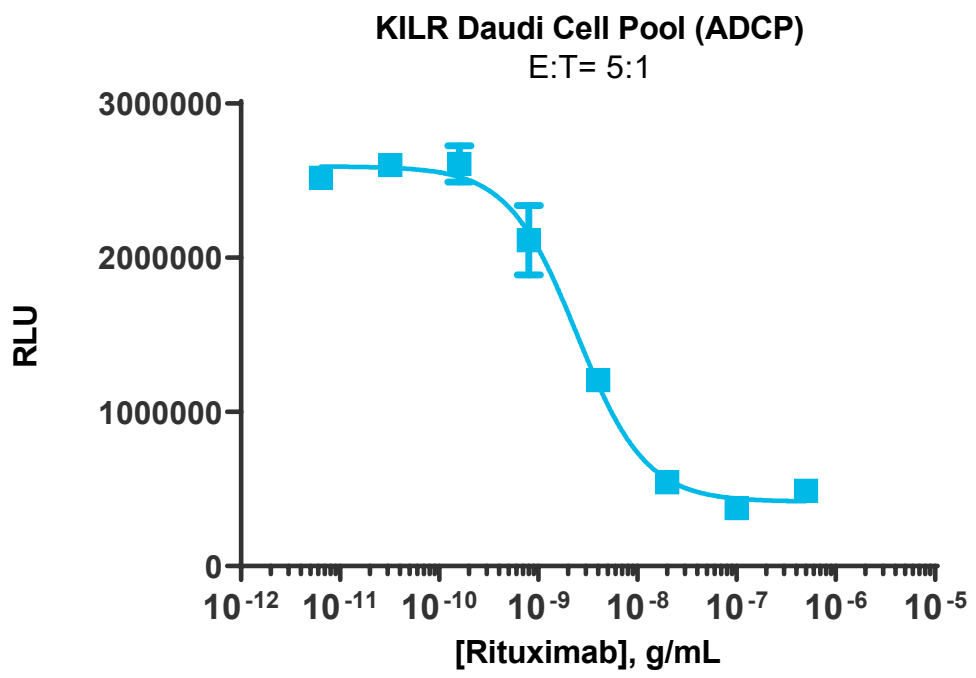
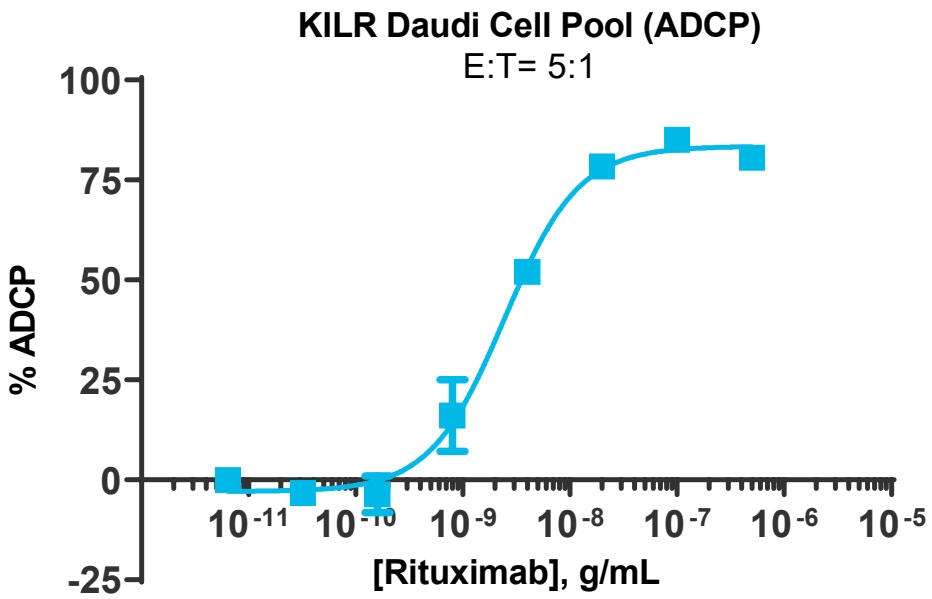
Raw Data



% 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

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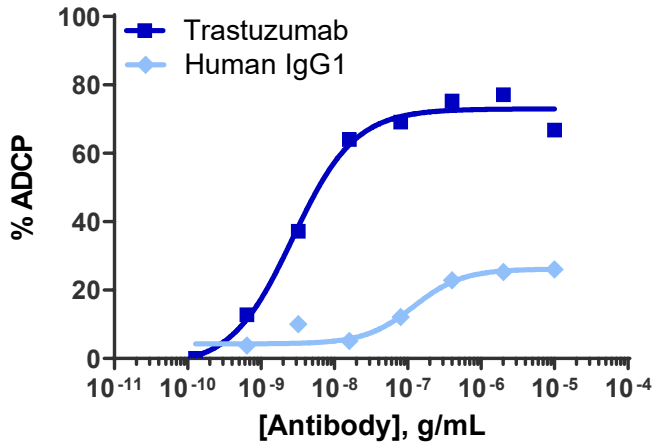
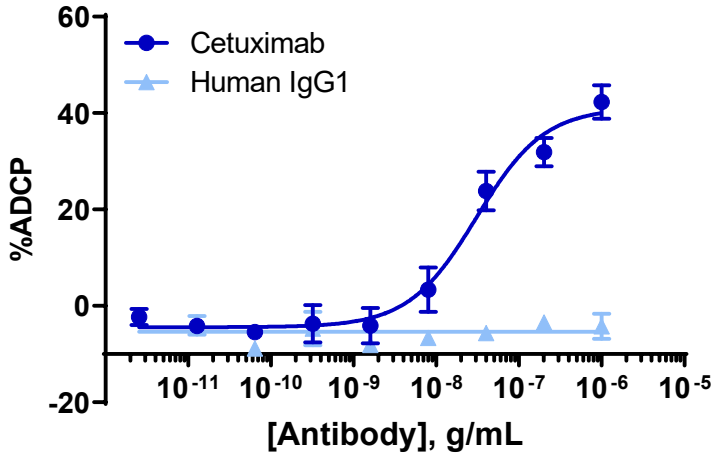
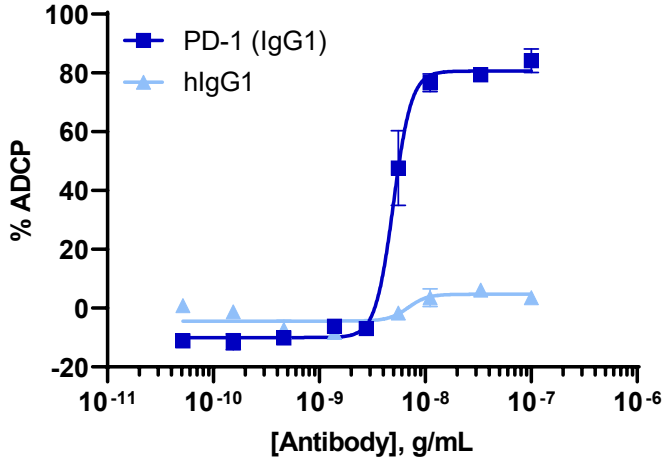
HER2+ Breast Cancer



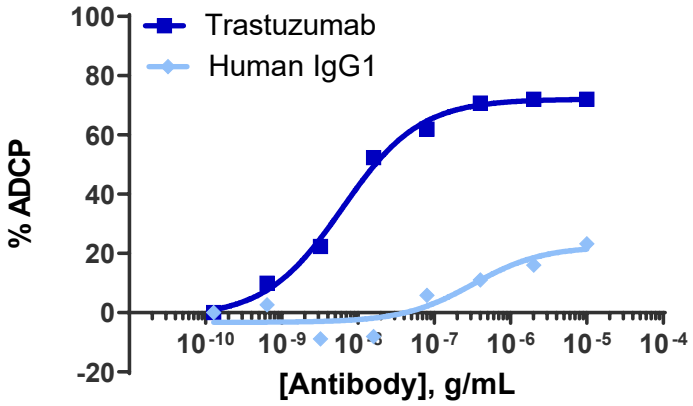
HER2- Breast Cancer



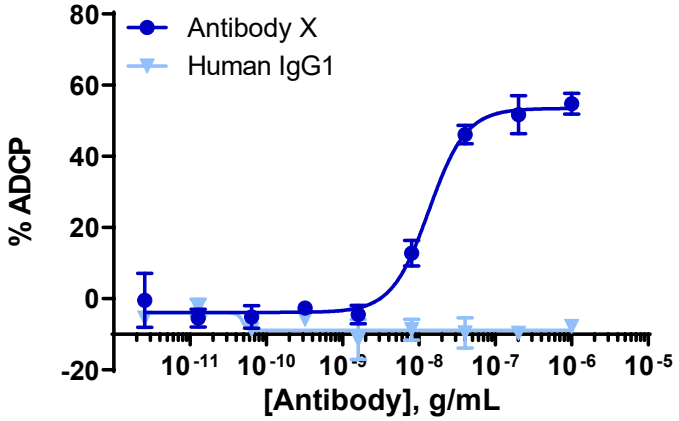
PD-1+ T Cell Model



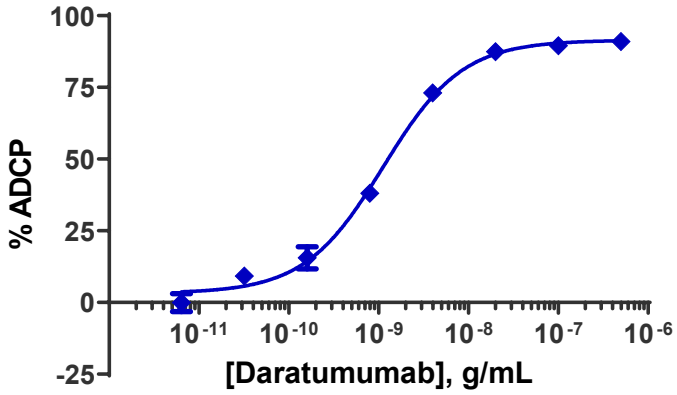
HER2+ Gastric Cancer



HER2- Breast Cancer



CD38+ B Cell Model



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

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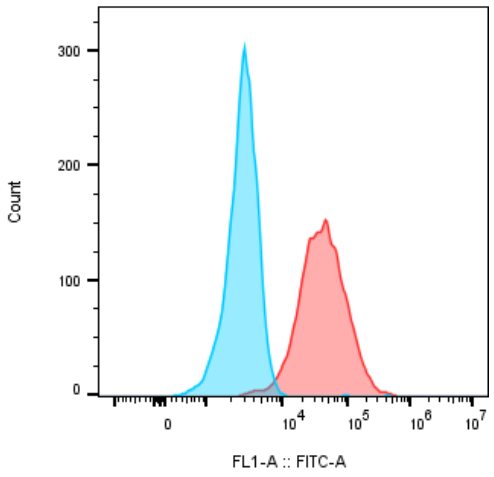
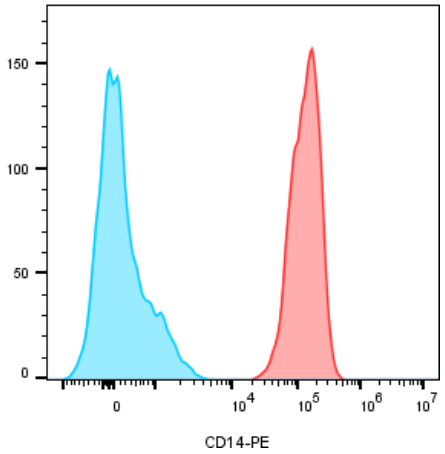
M0 Macrophage Markers

CD11b

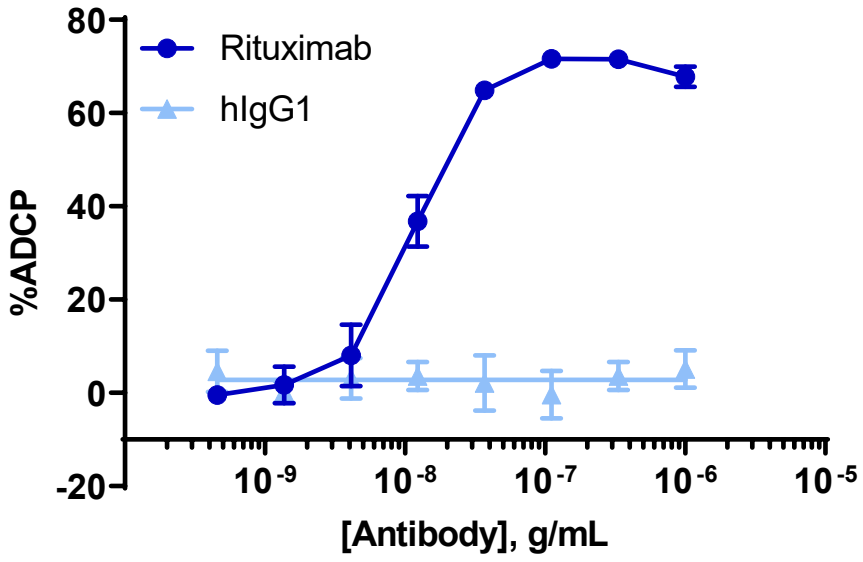


CD14

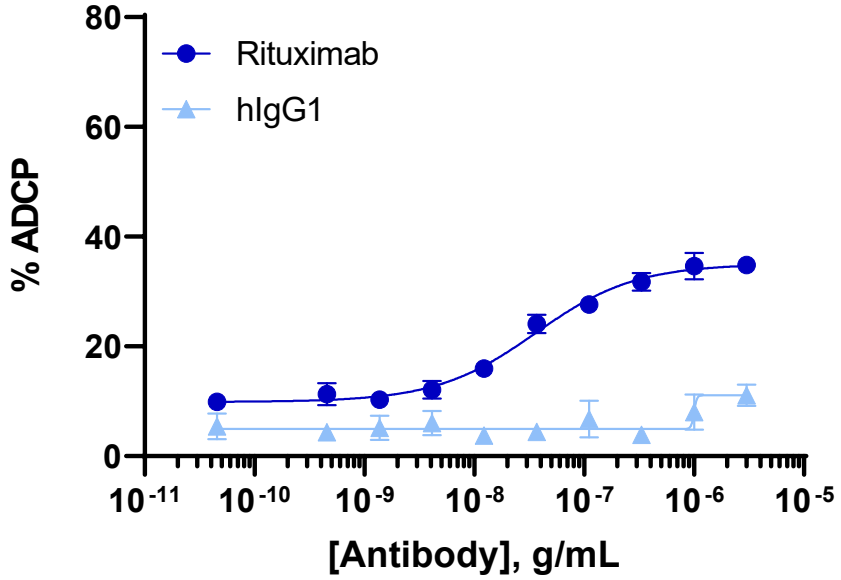


ADCP Results

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

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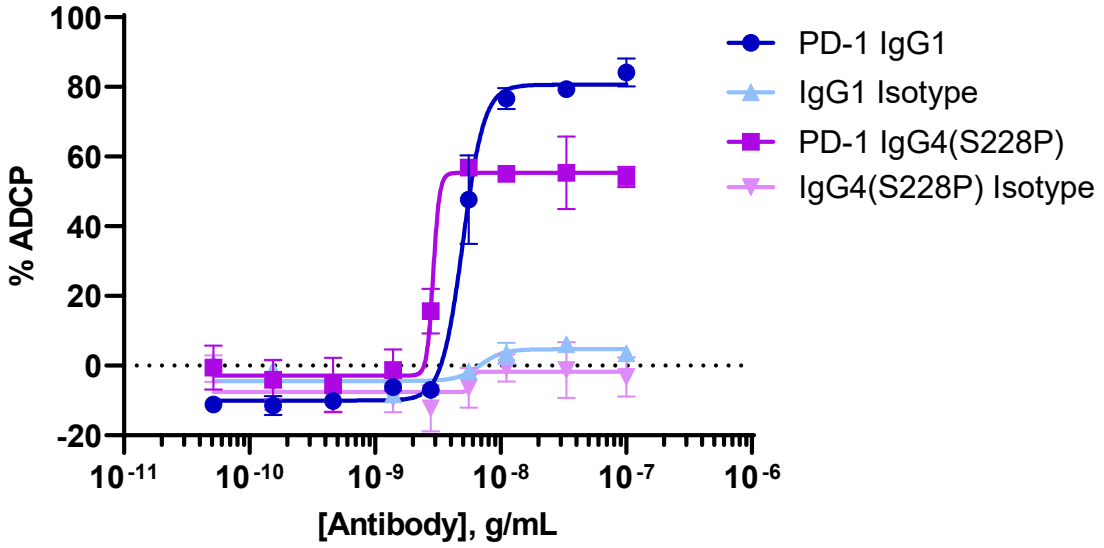
IgGx Affinity for Fc Receptors
(impact on immune-mediated killing)

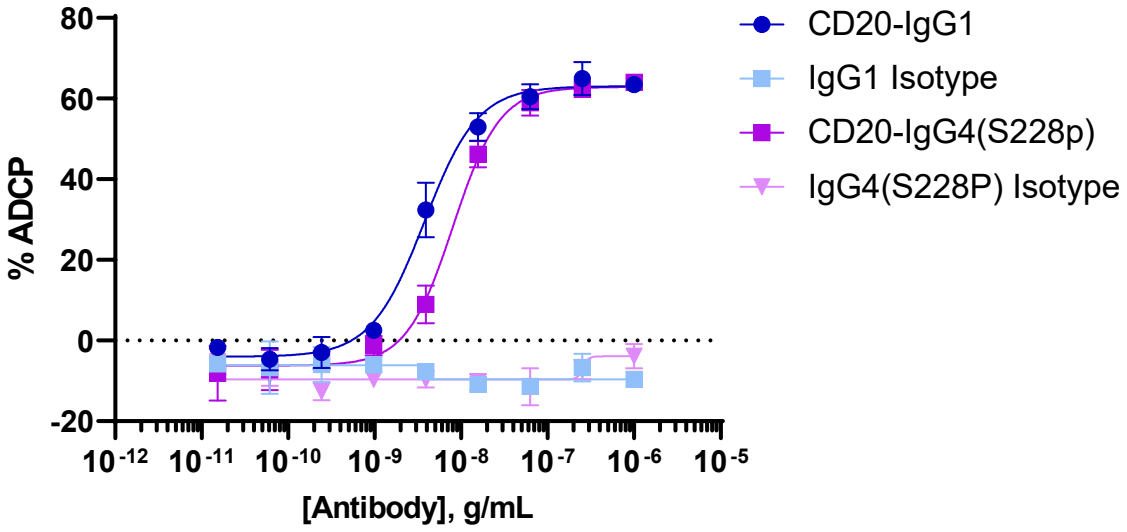
ADCC: IgG1 ≥ IgG3 > IgG2 ≥ IgG4
CDC: IgG3 > IgG1 > IgG2 > IgG4
ADCP: IgG1 ≥ IgG3 > IgG2 > IgG4

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

PD-1 Model



CD20 Model



Results indicate developers using an IgG4(S228P) Fc should evaluate ADCP activity of their therapeutics, preferably in multiple donors

Summary and Conclusions

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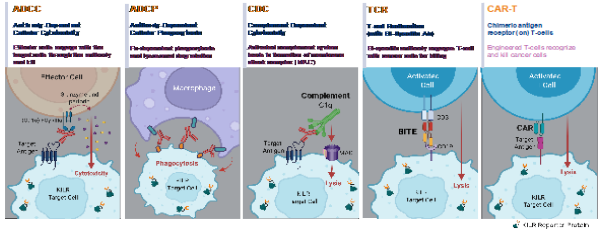
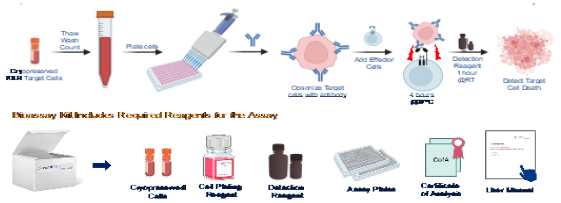
KILR® cytotoxicity platform offers MOA-based assays for variety of cell-mediated cytotoxicity applications such as:

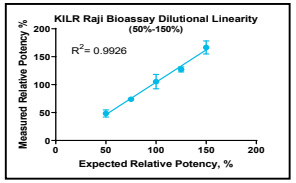
ADCC, ADCP, CDC, T-cell Redirection and CAR-T



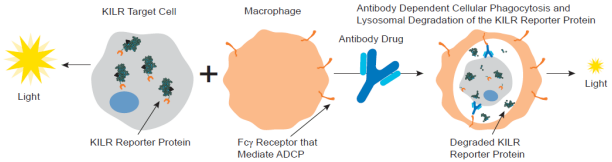
KILR ADCC Bioassays allows discrimination of differences in Fab and Fc regions to enable rank ordering of antibodies



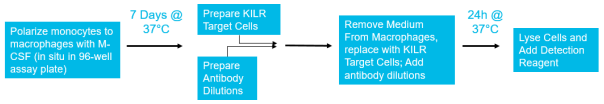
KILR ADCC Bioassays, when used in combination with KILR CD16 Effector Cells, produce highly accurate and precise data suitable for use in relative potency assays



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