



# Implementing MOA-Reflective ADCC assays using Ready-to-Use KILR<sup>®</sup> Target & Effector Cells from Screening to Lot Release

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Scientific Development Manager

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

*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

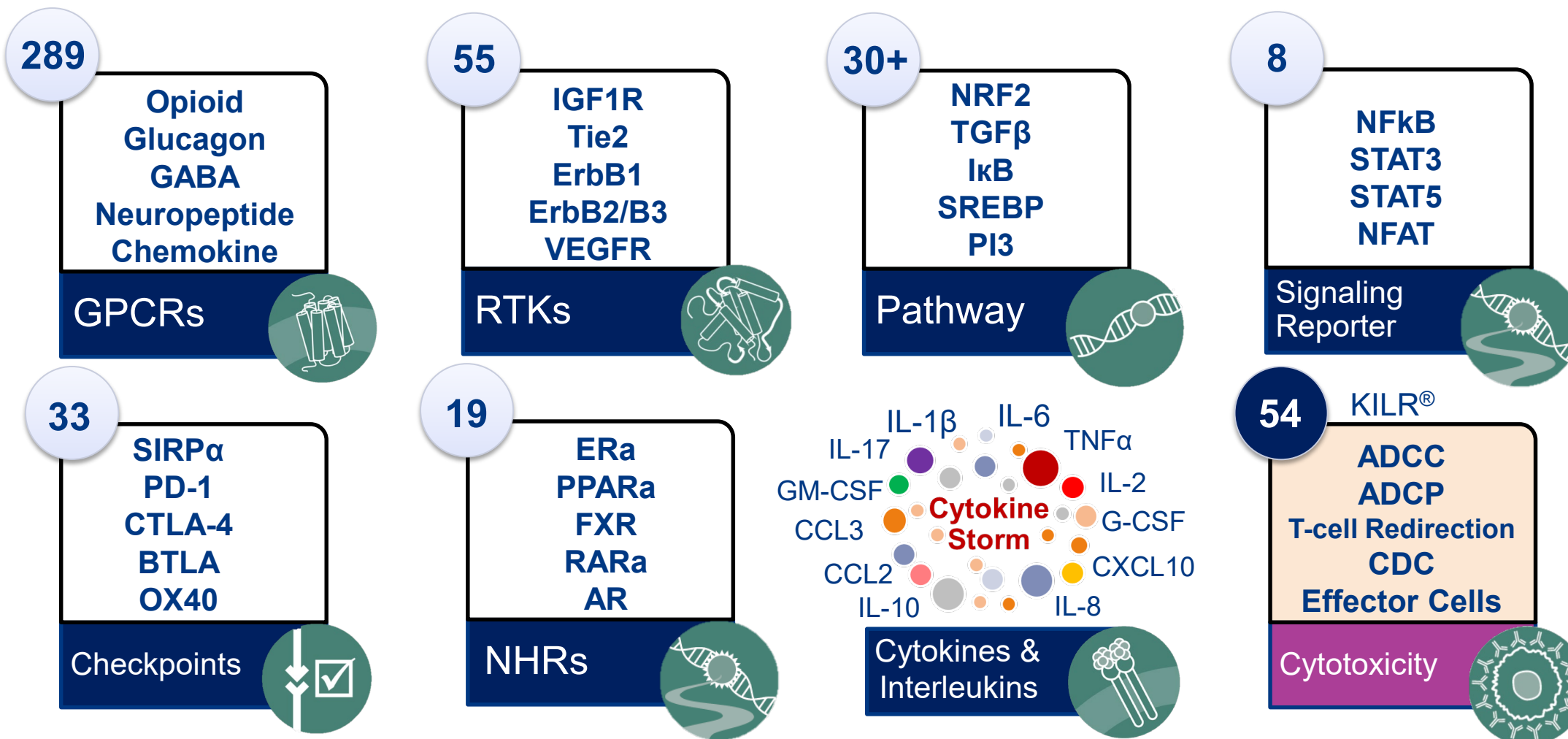
### 70+ Global Programs

For potency, stability and NAb  
testing

MOA = Mechanism-of-Action; ICH = International Council for Harmonization; CRO = Contract Research Organizations; NAb = Neutralizing Antibody

# Largest Menu of Cell-Based Assays for Discovery, Potency, & NAb Assay Development

>800 Cell lines to support bioassay development for major drug target classes



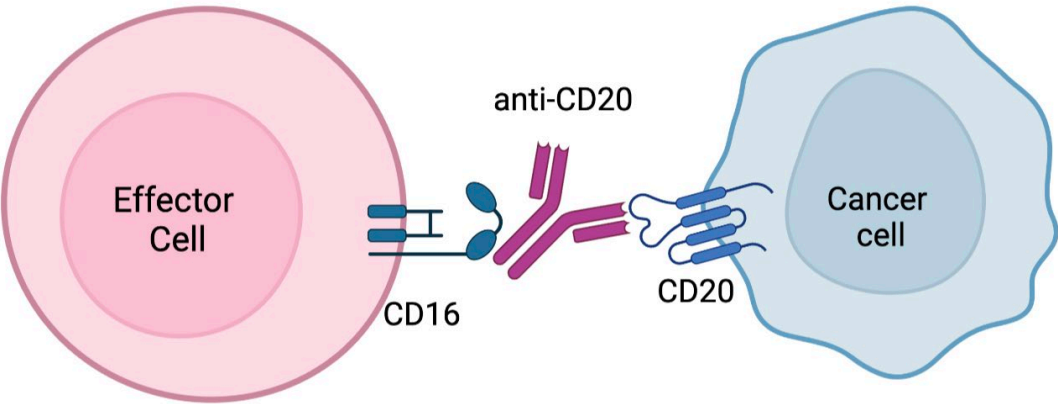
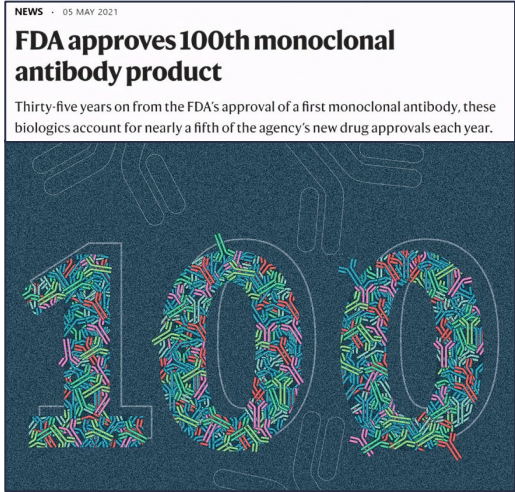


# Cell-Mediated Cytotoxicity – A Rapidly Developing Area For Cancer Therapeutics

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

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

Starting from Phase I and even earlier, ADCC assays should be performed for assessment of 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

- *KILR<sup>®</sup> assays are non-radioactive and are 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”*

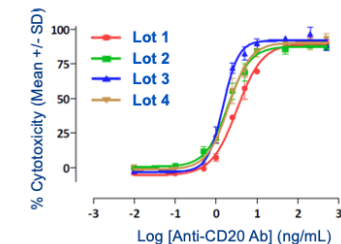
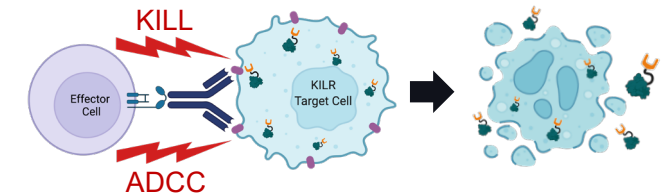
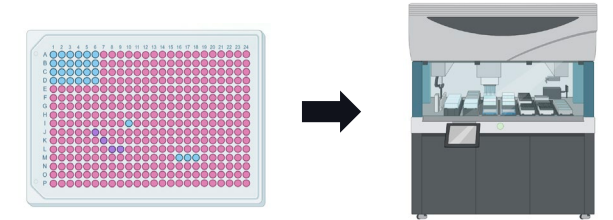
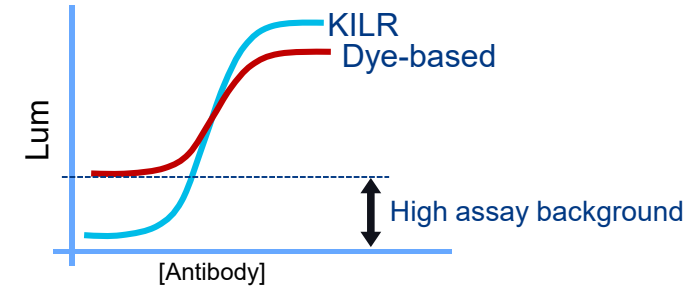
- *KILR assays are end-point assays and specifically measures target cell killing*

*Physiologically-reflective of TRUE MOA*

High donor-to-donor variability associated with effector cells

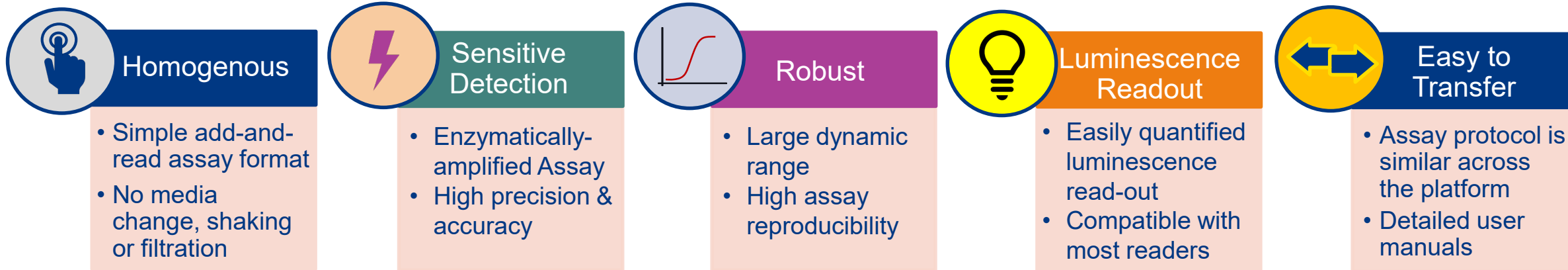
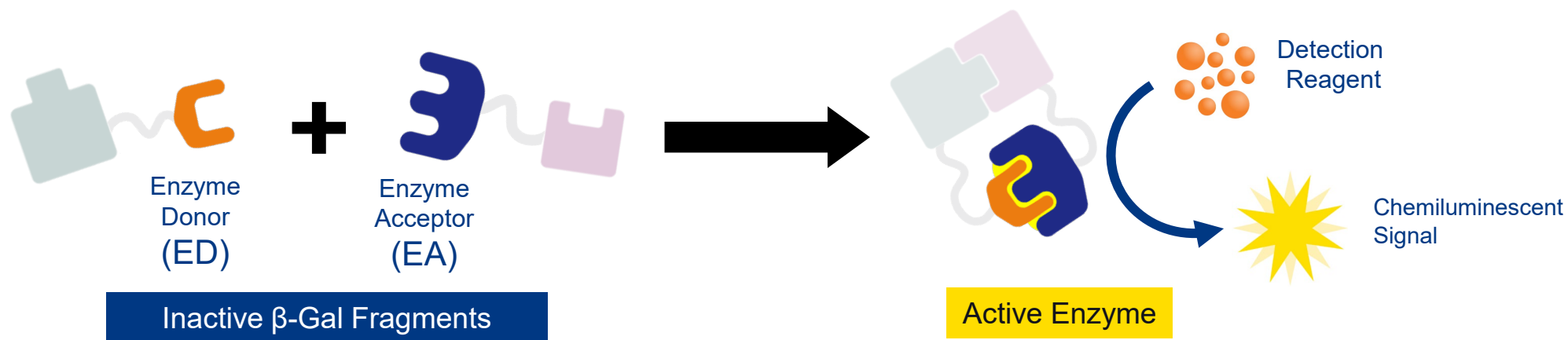
- *KILR CD16 Effector Cells are single donor-derived*

*Eliminate donor variability*



# Enzyme Fragment Complementation (EFC) Technology

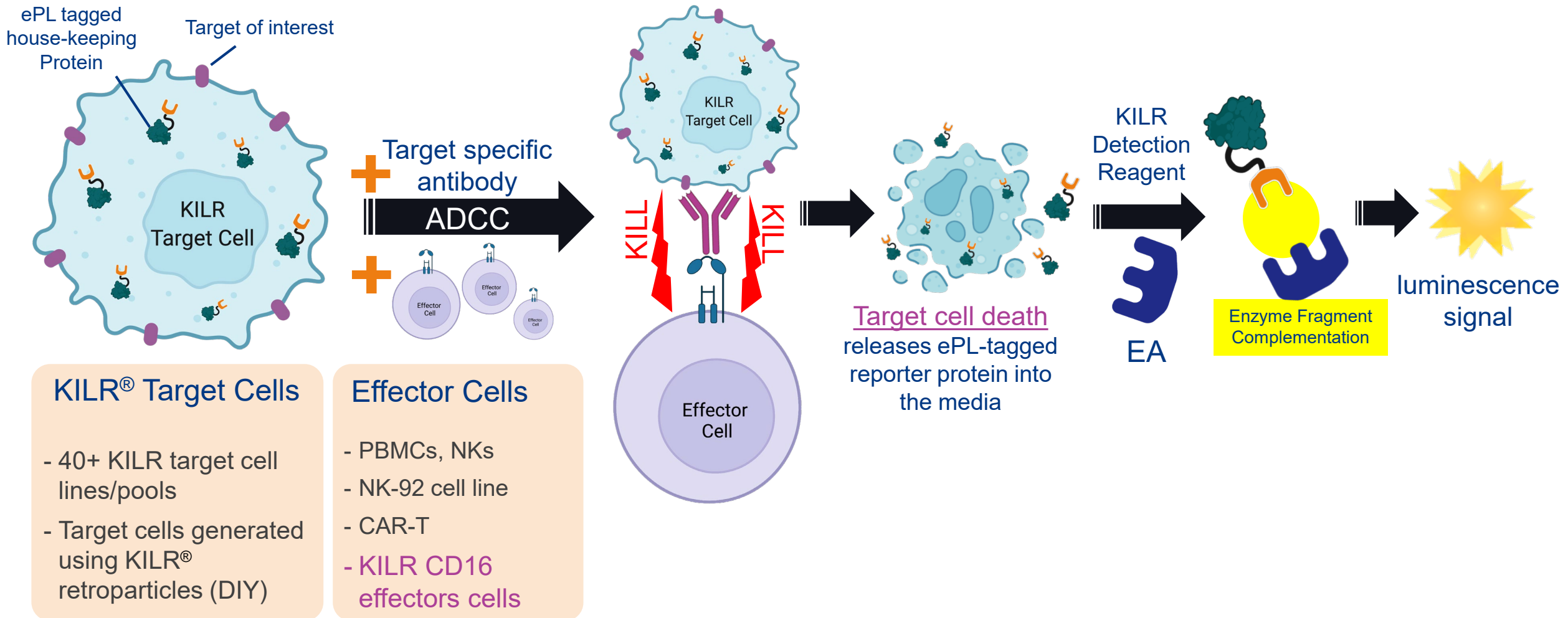
*Enabling Technologies with a Flexible Platform based on a Split  $\beta$ -Galactosidase Enzyme*



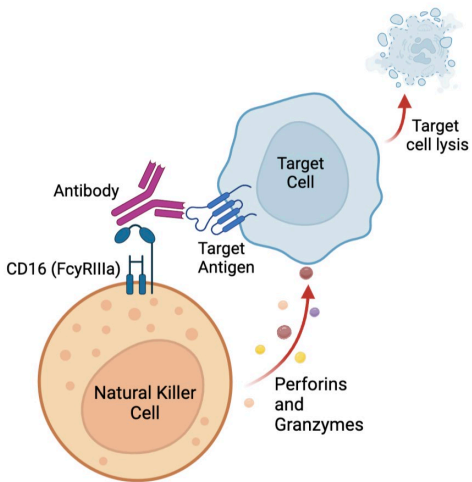
# KILR Cytotoxicity Assay Overview

## ADCC Example

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

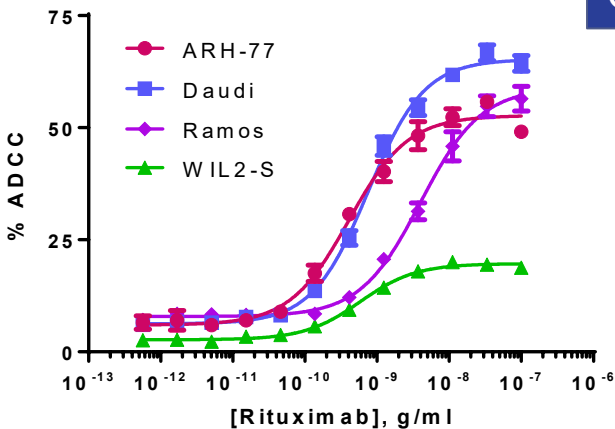


# ADCC Demonstrated Using KILR<sup>®</sup> Assay with a Variety of Antibodies, Antigens, and Cell Types



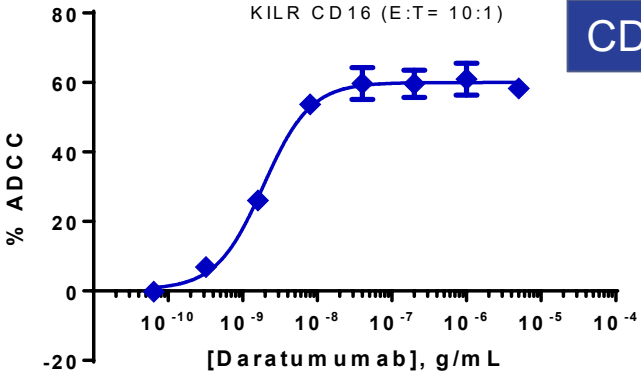
**CD20+ KILR Cell Pools**  
PBMCs (E:T= 25:1)

CD20



**KILR Raji Cell Pool**  
KILR CD16 (E:T= 10:1)

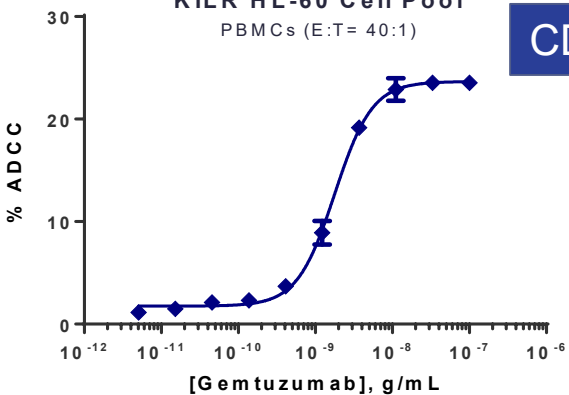
CD38



Sample	E <sub>Max</sub>	EC <sub>50</sub> , ng/mL
Daratumumab	60%	1.89

**KILR HL-60 Cell Pool**  
PBMCs (E:T= 40:1)

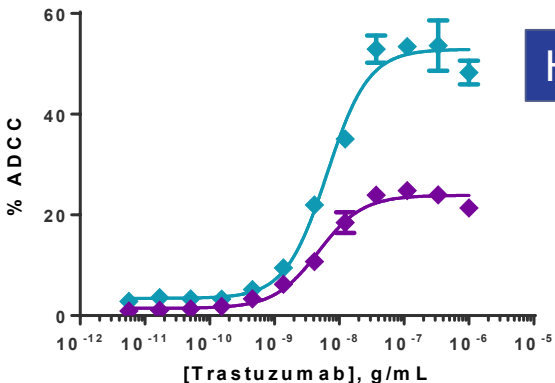
CD33



Sample	E <sub>Max</sub>	EC <sub>50</sub> , ng/mL
Gemtuzumab	24%	1.78

**KILR SKBR3 Cell Pool**  
Effector (PBMC): Target= 25:1

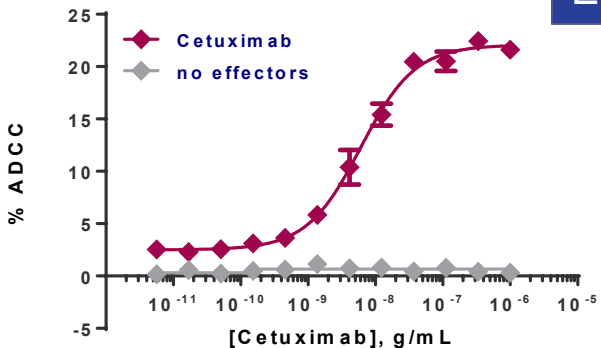
HER2



Sample	E <sub>Max</sub>	EC <sub>50</sub> , ng/mL
Trastuzumab (5K, 10K)	53.6%, 24.8%	4.74, 6.44

**KILR NCI-N87 Cell Pool**  
PBMCs (E:T= 25:1)

EGFR



Sample	E <sub>Max</sub>	EC <sub>50</sub> , ng/mL
Cetuximab	22%	6.04



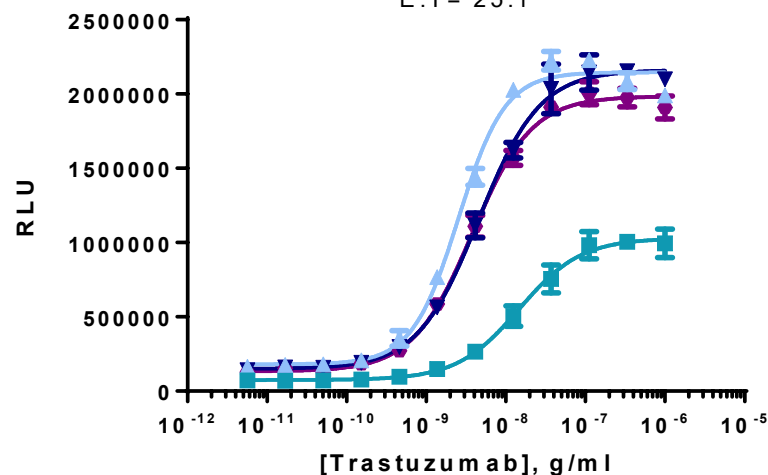
# KILR® ADCC Assay is Compatible with Multiple Effector Cell Types

Primary PBMCs and NKs, or NK-92 (CD-16)

## Primary PBMCs

### KILR SKBR3 Cell Pool

FcγRIIIa V158 vs F158 homo- or heterozygotes  
E:T = 25:1

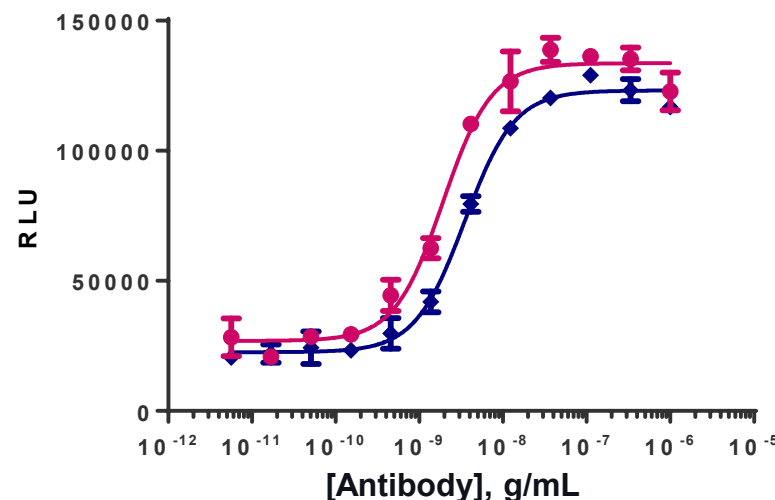


Donor	S/B	HillSlope	EC <sub>50</sub> , ng/mL
#226 (F/F)	13.7	1.113	14.6
#337 (F/V)	13.9	1.486	2.53
#74 (V/V)	15.2	1.128	4.54
Pool	15.5	1.161	3.76

## Primary NK cells

### KILR SKBR3 Cell Pool

Effector (NK):Target Ratio = 4:1

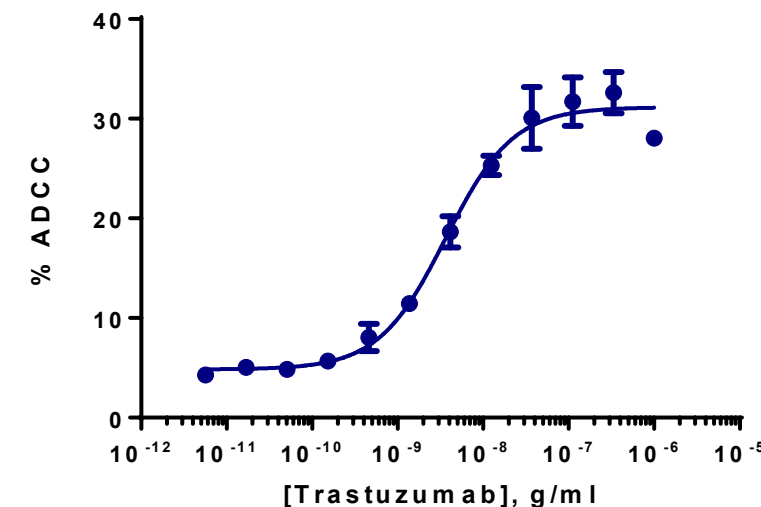


Sample	S/B	HillSlope	EC <sub>50</sub> , ng/mL
Trastuzumab	5.0	1.501	1.92
Pertuzumab	5.5	1.463	3.48

## NK-92 (CD-16)

### KILR SKOV-3 Cell Pool

Effector (NK-92): Target = 10:1

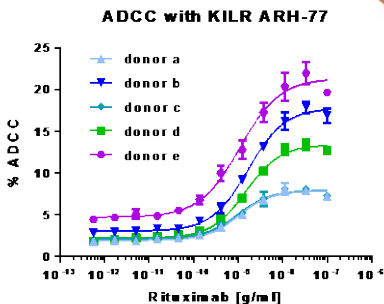
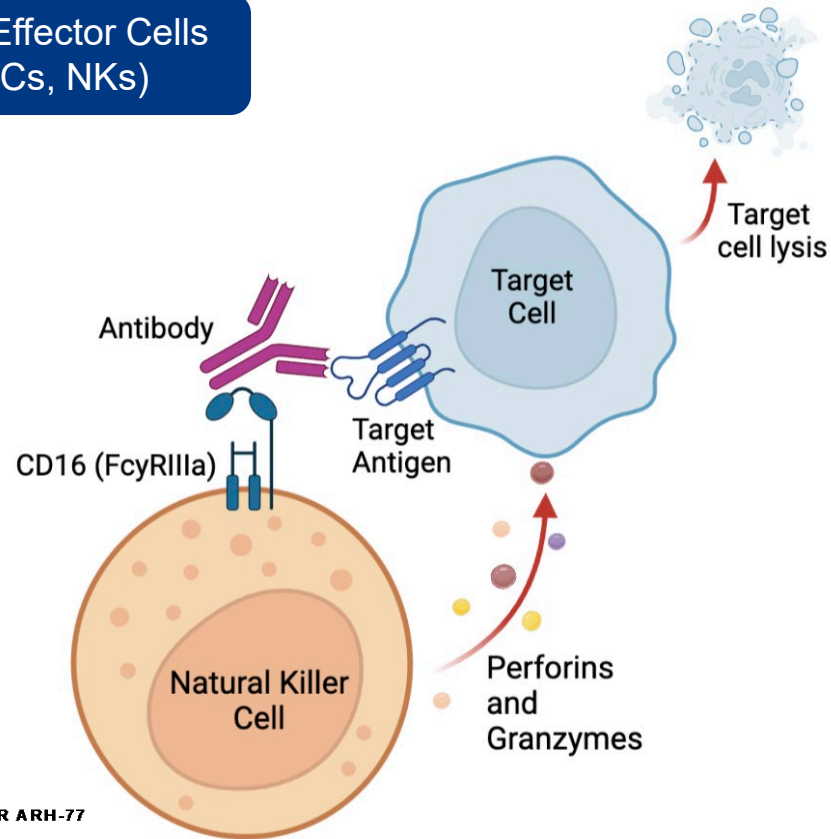


Sample	S/B	HillSlope	EC <sub>50</sub> , ng/mL
Trastuzumab	7.6	1.107	3.63

# Eliminating Donor Variability in ADCC Assays

## KILR® CD16 Effector Cells

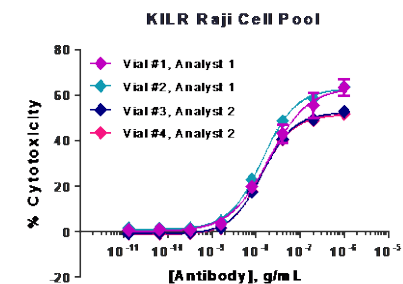
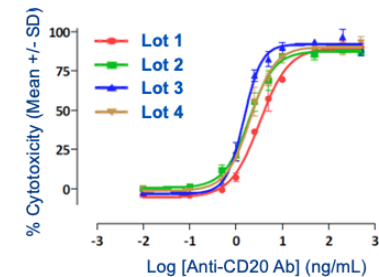
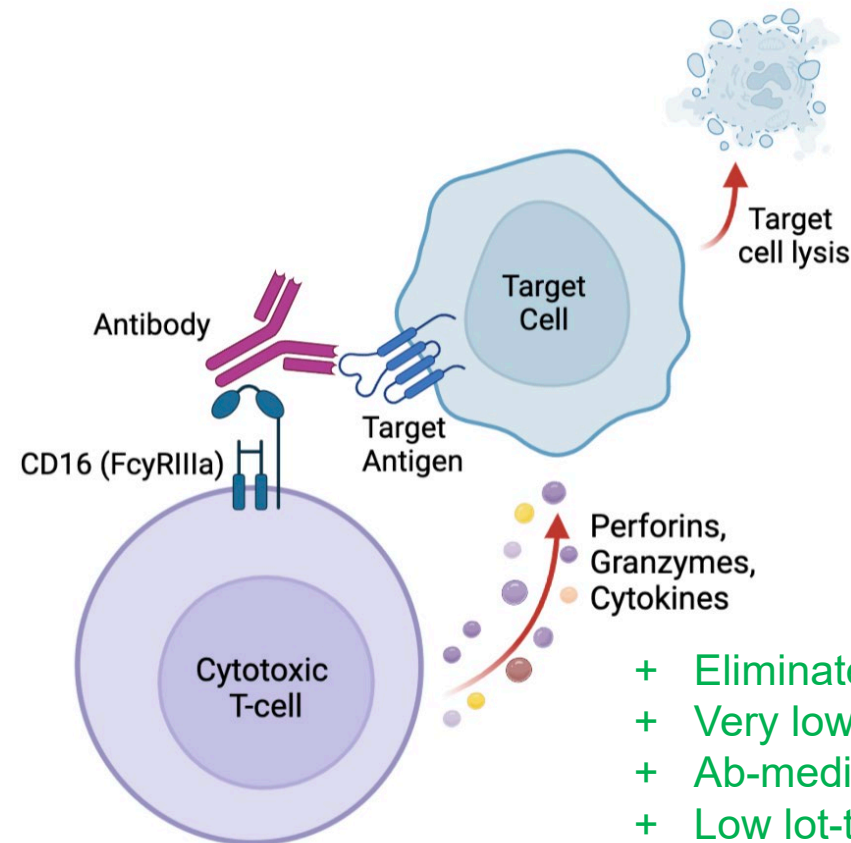
Isolated Effector Cells  
(PBMCs, NKs)



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

KILR CD16 Effector Cells

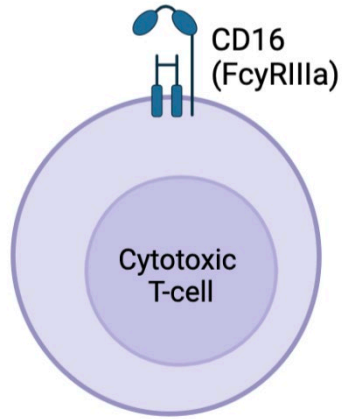
Single donor-derived Cytotoxic T cells  
engineered to express CD16 receptor



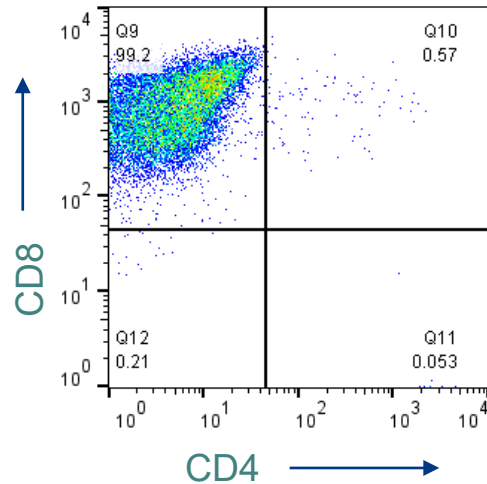
- + Eliminates donor variability
- + Very low background killing
- + Ab-mediated rapid killing kinetics
- + Low lot-to-lot variability

# Single Donor-Derived Engineered Effector Cells for ADCC

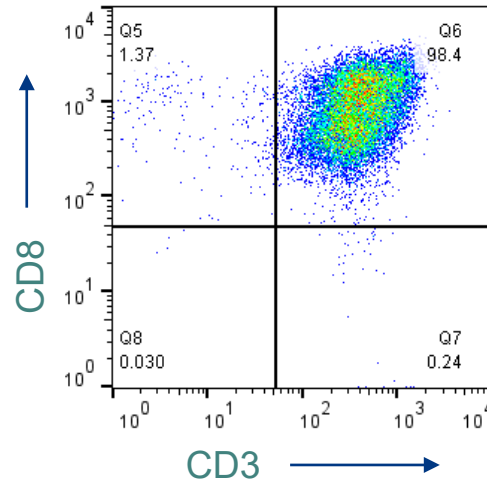
## KILR® CD16 (V158) Effector Cells



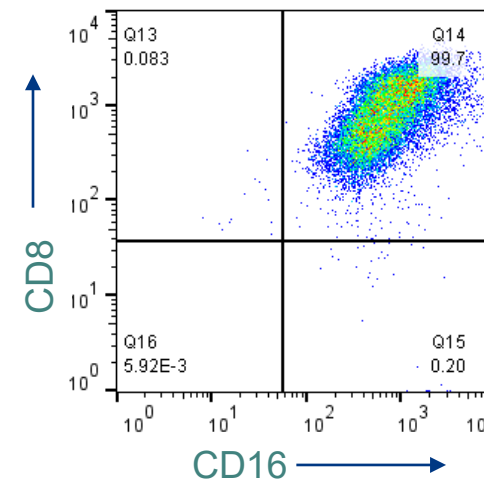
- Primary human cytotoxic T lymphocytes (CTLs)
- Engineered to stably express CD16 (V158)
- Uniformly manufactured to ensure lot-to-lot consistency
- Low background, high killing capacity for implementing ADCC for lot release or characterization



Polyclonal population, 99% CD8<sup>+</sup>



98% CD3<sup>+</sup> / CD8<sup>+</sup>

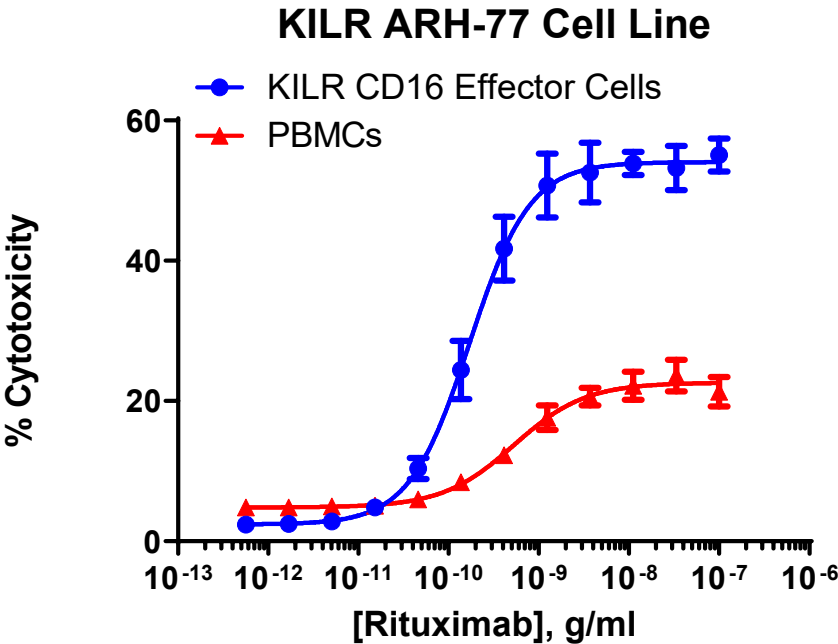


Stable CD16 expression (99.7%)

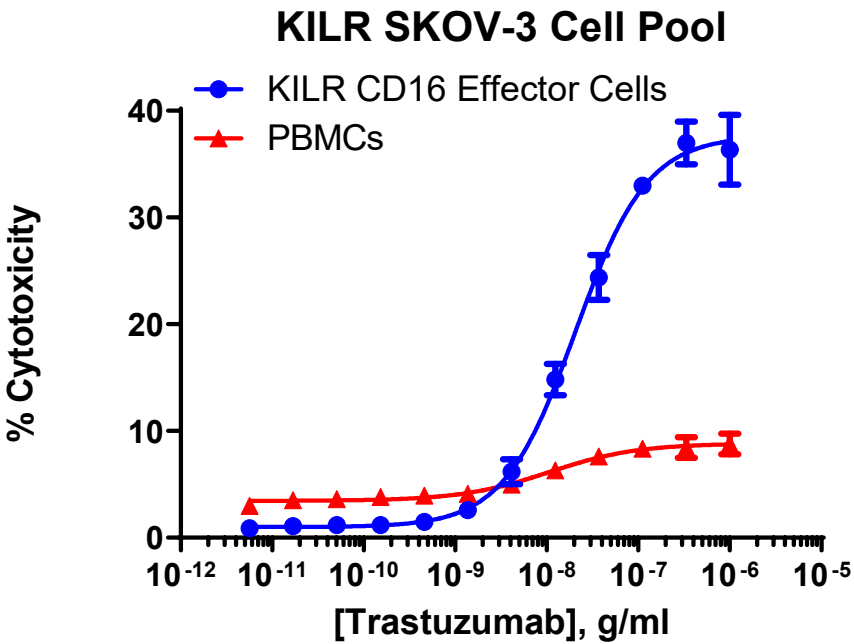
# Larger Assay Window Improves Analysis of Antibody Activity

## ADCC Assays

Consistent pharmacology with lower effector:target ratios



Sample	EC <sub>50</sub> , ng/mL	S/B	Max Cytotoxicity, %
KILR® CD16 cells (10:1)	0.17	23	54%
PBMCs (25:1)	0.53	5.5	22.6%



Sample	EC <sub>50</sub> , ng/mL	S/B	Max Cytotoxicity, %
KILR CD16 cells (12.5:1)	20.6	41.6	37%
PBMCs (25:1)	10.6	2.6	8.8%

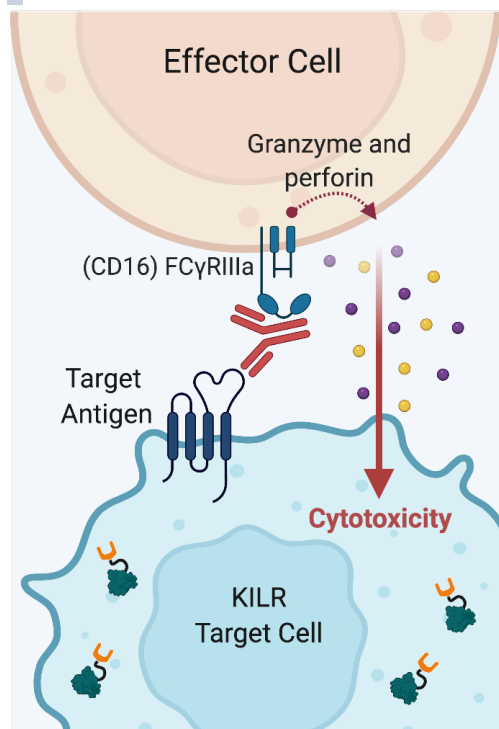


# MOA's Supported by KILR<sup>®</sup> Cytotoxicity Assays

## ADCC

### Antibody-Dependent Cellular Cytotoxicity

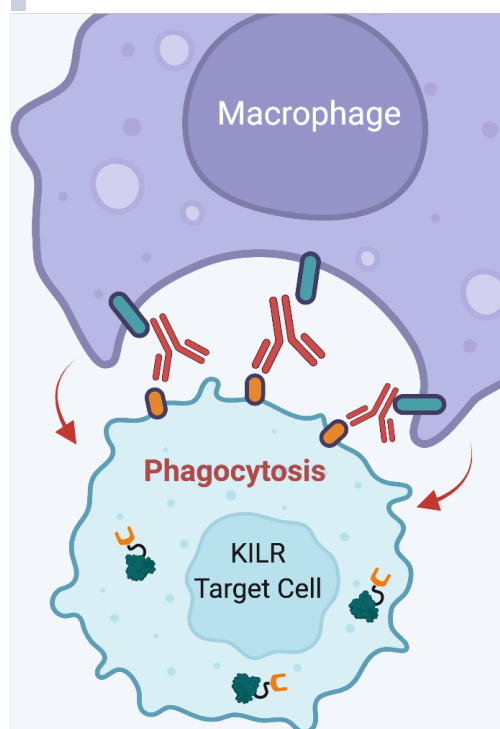
Effector cells engage with the target cells through the antibody and kill



## ADCP

### Antibody-Dependent Cellular Phagocytosis

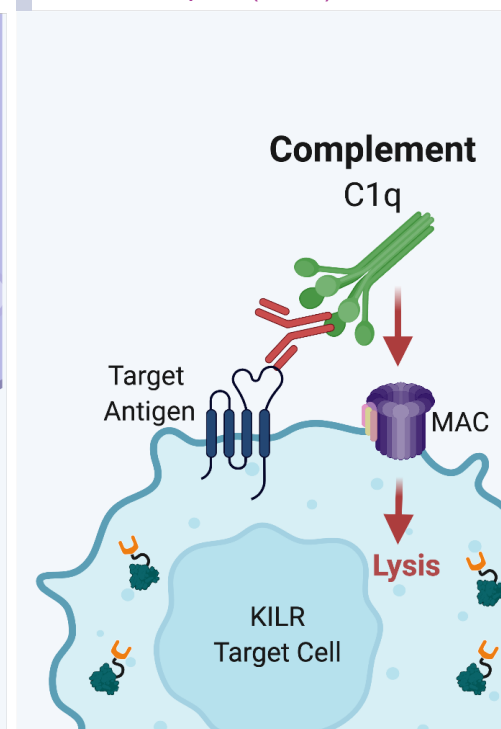
Fc-dependent phagocytosis and lysosomal degradation



## CDC

### Complement Dependent Cytotoxicity

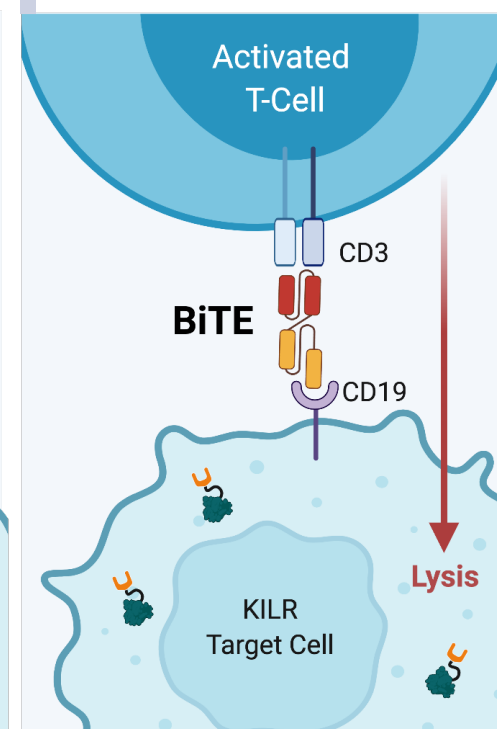
Activated complement system leads to formation of membrane attack complex (MAC)



## TCR

### T-cell Redirection (with Bi-Specific Ab)

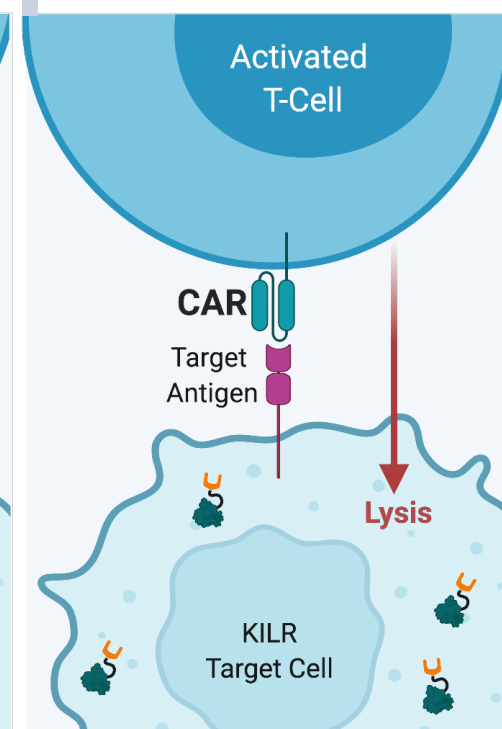
Bi-specific antibody engages T-cell with cancer cells for killing



## CAR-T

### Chimeric antigen receptor (on) T-cells

Engineered T-cells recognize and kill cancer cells

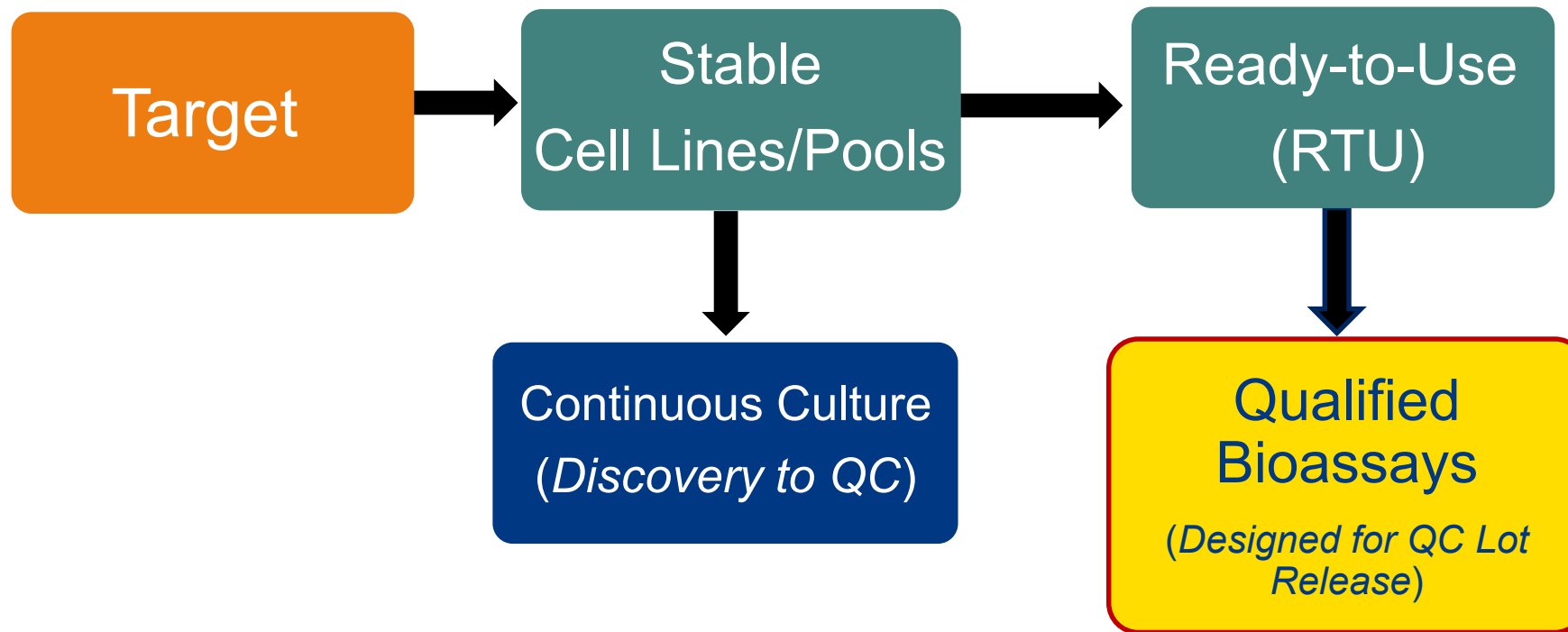


KILR Reporter Protein

# Flexible Assay Format

## Phase-Appropriate Solutions for Every Development Program

*From Continuous Culture to Qualified Bioassays*



**LUCENTIS**  
RANIBIZUMAB INJECTION

**Vectibix**  
(panitumumab)  
Injection for IV Infusion

**KEYTRUDA**

**ACTEMRA**  
tocilizumab

**AVASTIN**  
bevacizumab  
100MG/4ML INJECTION FOR IV USE

**OPDIVO**  
(nivolumab)

**EYLEA**  
aflibercept solution for injection

**NovoLog**  
insulin aspart (rDNA origin) injection

**VICTOZA**  
liraglutide injection 1.2mg/1.8mg

**GONAL-f**  
FOLLITROPIN ALFA

**Byetta**  
exenatide injection

**Aranesp**  
darbepoetin alfa  
injection

**PERJETA**  
pertuzumab for injection

**Humalog**  
insulin lispro

**FORTEO**  
teriparatide (rDNA origin) injection

**Kineret**  
(anakinra)

**Leukine**  
sargramostim  
A Recombinant GM-CSF-Yeast-Expressed

**HUMIRA**  
adalimumab

**Erythropoietin**

**HGH** SOMATROPIN  
Recombinant Human Growth

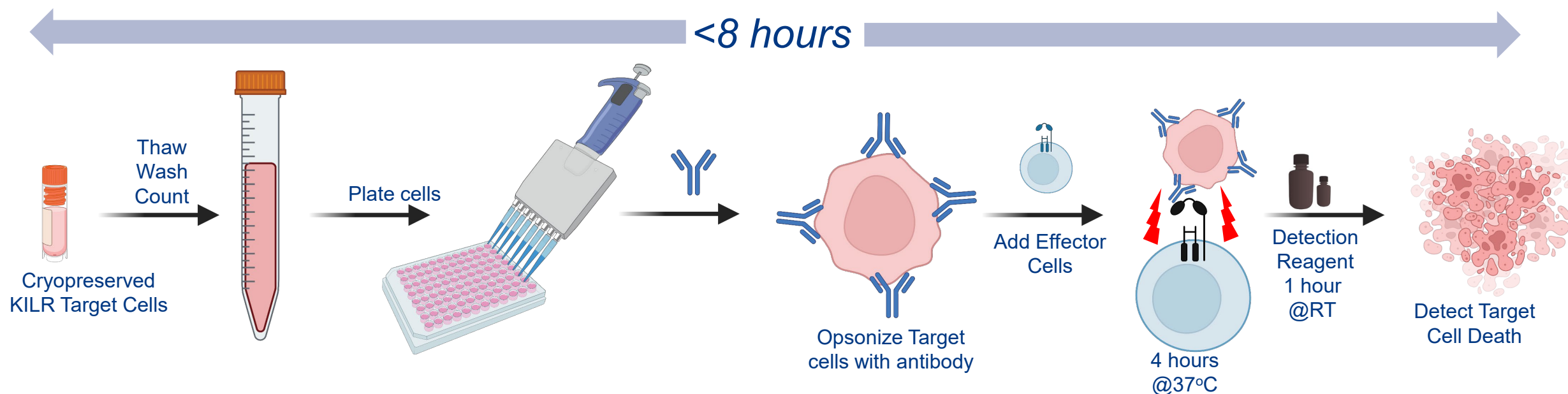
## Launching Today - KILR<sup>®</sup> Raji ADCC Bioassay Kit

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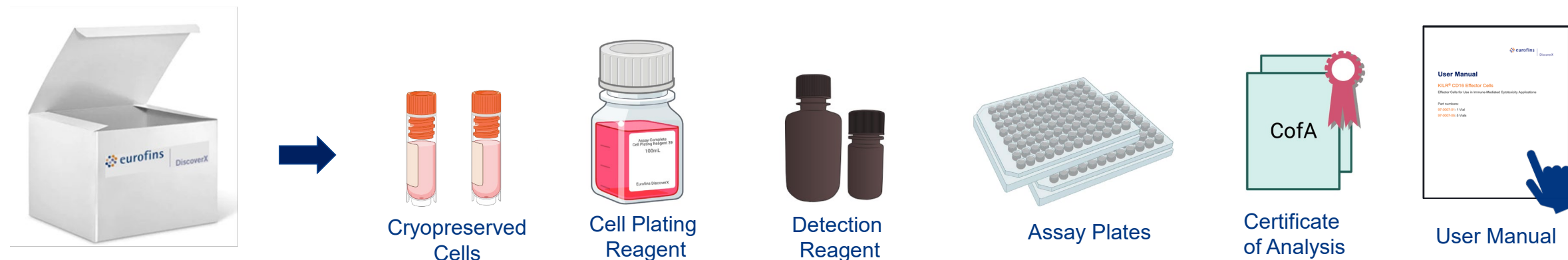
QC = Quality Control

# Easy-to-Use Assay Protocol

## KILR<sup>®</sup> Raji ADCC Bioassay Kit



## Bioassay Kit Includes Required Reagents for the Assay



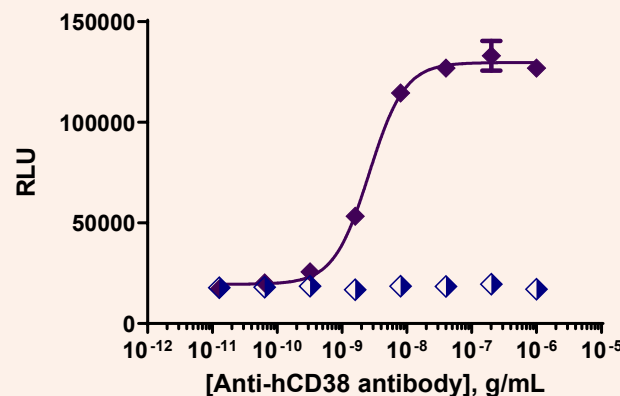
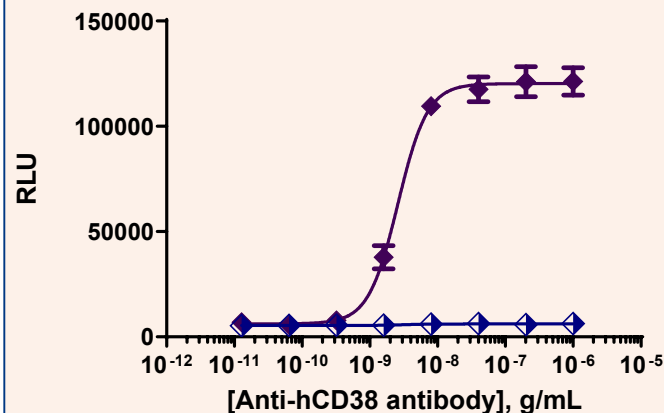
# Continuous Culture vs Ready-to-Assay Cell Formats

## KILR® Raji ADCC Bioassay Kit Performance

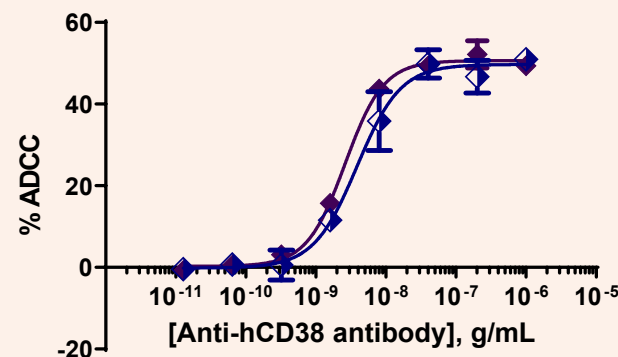
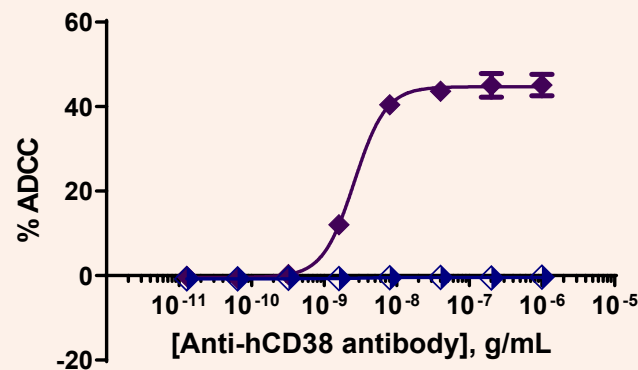
### Continuous Culture

### Ready-to-Assay

#### Raw Luminescence (RLU)



#### %Killing (%ADCC activity)



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

*Excellent Concordance between EC<sub>50</sub>*

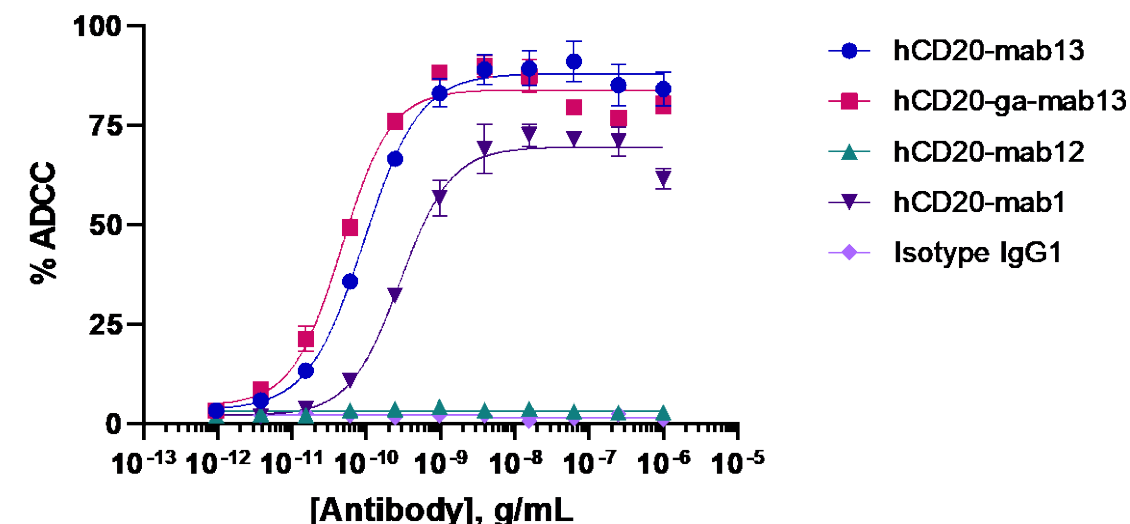
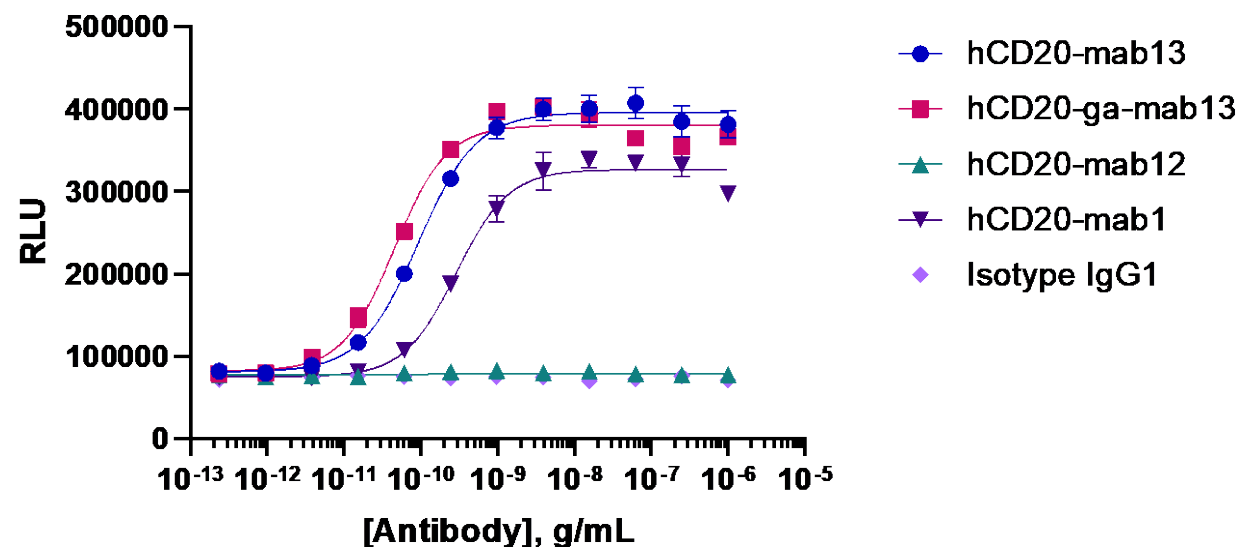
Condition	E <sub>Max</sub>	EC <sub>50</sub> , ng/mL
Continuous Culture	45%	2.6
Assay-ready	51%	2.6

*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 <sub>Max</sub>	EC <sub>50</sub> , 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 identifies subtle differences to differentiate to obtain potency rank order*

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

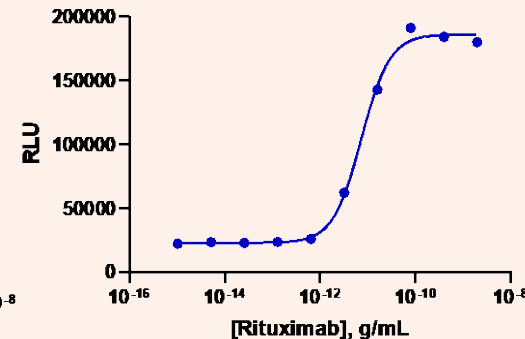
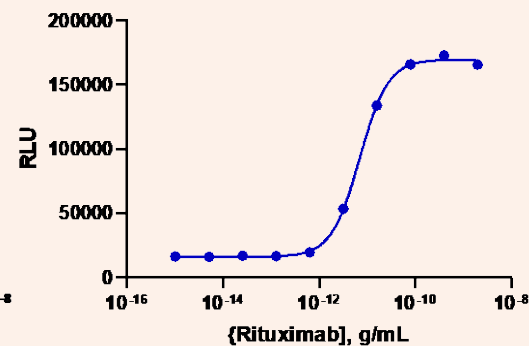
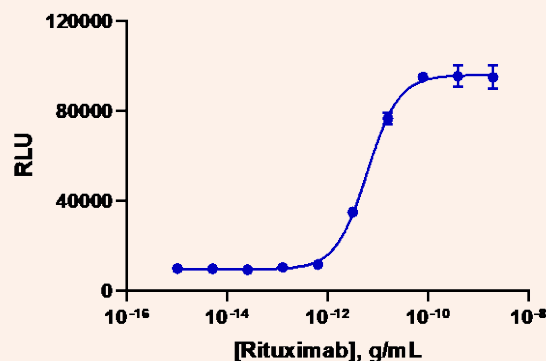
Day 1

Day 2

Day 3

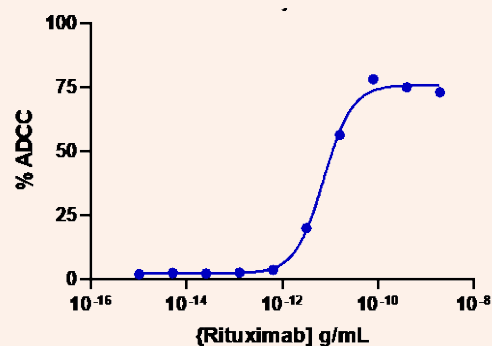
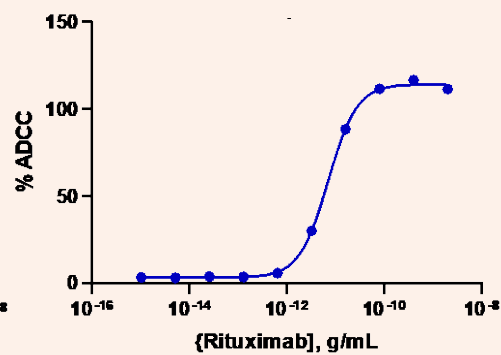
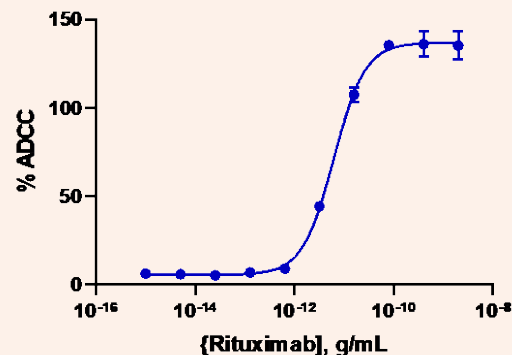
Raw Luminescence (RLU)

Is



%Killing (%ADCC activity)

i



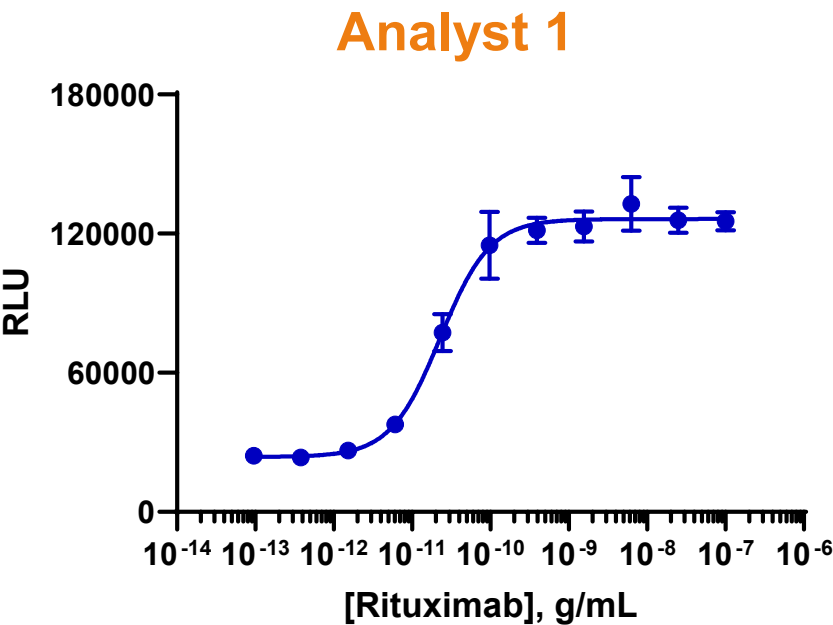
Parameter	Day 1	Day 2	Day 3	% RSD
EC <sub>50</sub> , pg/mL	6.2	7.03	7.4	8.9%
S/B	9.8	10.6	8.3	12.2%
E <sub>Max</sub> , %	135%	114%	75.8%	27.7%

*Excellent inter-day reproducibility*

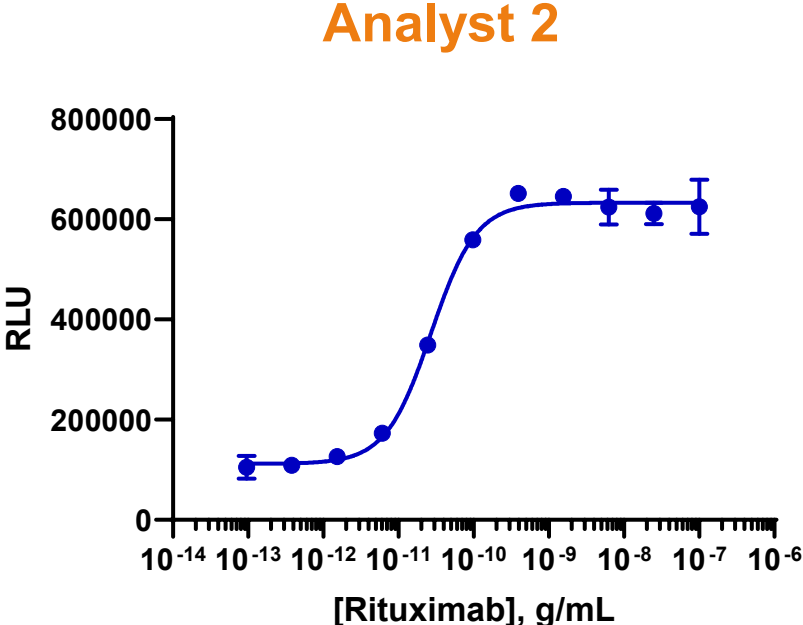
# Comparable Inter-Analyst Data

## KILR<sup>®</sup> Raji ADCC Bioassay Kit

Independent experiments performed on different days with same Bioassay cells from same lot




S/B	HillSlope	EC <sub>50</sub> , pg/mL
4.9	1.375	22.9



S/B	HillSlope	EC <sub>50</sub> , pg/mL
5.9	1.448	27.1

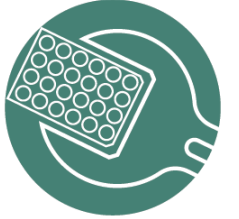
Consistent assay performance between individual analysts

# Global Programs using DiscoverX Bioassays for Potency, Stability, & NAb Testing for Drug Release

<b>SIRPα Bioassay</b> Multiple Programs in NA for originator biologics NAb assessment Programs at CROs	<b>PD-1 Bioassay</b> Multiple Programs in NA and APAC Clinical Development of Biosimilars	<b>VEGF Bioassay</b> Multiple Programs in NA, EU and APAC Clinical Development of Biosimilars	<b>Insulin Bioassay</b> Multiple Programs in and APAC for clinical Development of Biosimilars	<b>CNR1 Bioassay</b> US based Biopharma for originator biologic	<b>GLP1R Bioassay</b> Multiple Programs in NA, EU and APAC Clinical Development of Biosimilars in NA	<b>IGF1R Bioassay</b> Multiple Programs in NA and APAC for originator biologic	<b>Bradykinin Bioassay</b> US based CRO for undisclosed client	<b>PTHR1 Bioassay</b> Multiple Programs in NA, EU and APAC Clinical Development of Biosimilars
<b>ErbB2/ErbB3 Bioassay</b> Multiple Programs in NA, EU and APAC Clinical Development of Biosimilars	<b>FGFR Bioassay</b> NAb assessment program for US based Biopharma	<b>IL-1 Bioassay</b> Multiple Programs in NA for originator biologic				<b>Melanocortin Bioassay</b> European Biopharma for an originator biologic	<b>IL-2 Bioassay</b> Multiple Programs in NA and EU for originator biologic	<b>IL-23 Assay</b> US Based Biopharma for originator biologic
<b>IL-10 Bioassay</b> Multiple Programs in NA for originator biologic	<b>GM-CSF Bioassay</b> Multiple Programs in EU and NA for originator biologic	<b>IL-31 Bioassay</b> US based pharma for NAb assessment and potency testing Programs for originator biologic				<b>RANK Assay</b> Multiple Programs in and APAC for clinical Development of Biosimilars	<b>MC4R Bioassay</b> Bioassay Program in EU for originator biologic	<b>CXCR2 Bioassay</b> Bioassay Program in NA clinical development and drug release
<b>AXL Bioassay</b> Bioassay Program in NA for originator biologic	<b>CSF2R Bioassay</b> Multiple Programs in NA and EU Clinical development and drug release	<b>CSF1R Bioassay</b> Multiple Programs in NA and EU Clinical development and release				<b>KILR CD16 Effectors</b> Multiple Programs in NA and EU for Cytotoxicity Release assay	<b>FSHR Bioassay</b> Bioassay Program in APAC for Biosimilar	<b>IL7R/IL2R Bioassay</b> Bioassay Program in EU for originator biologic
			<b>CXCR4 Bioassay</b> NAb assessment program for EU based Biopharma	<b>IL7R/CRLF2 Bioassay</b> Drug Release Program in EU for originator biologic	<b>CALCRL/RAMP3 Bioassay</b> Bioassay Program in NA clinical development and drug release			

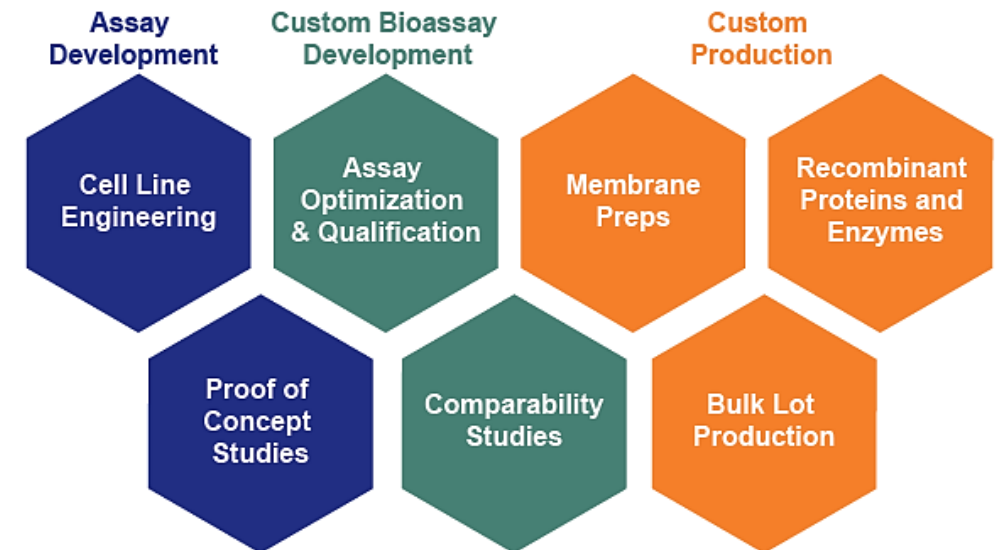


## *Your Target Biology, Our Expertise — Building a Better Assay Together*



- **Development Expertise** — Decades of cell-based assay development, cell line engineering, and recombinant enzyme development expertise
- **Cell Line Engineering Capability** — Exogenous expression approaches (constitutive vs inducible) or gene editing (e.g. KO/KI with CRISPR/Cas9)
- **Collaborative** — Consultative assay development with regular updates through a dedicated project manager
- **Complete Solution** — Customized assay development with screening and profiling services within the same team

### CAD Services Capabilities



# Multiple Key Capabilities, One Service Provider

## Eurofins Discovery Biotherapeutics Services

### Early Discovery

- Reagent generation
- Protein production
- Assay development



### Hit Finding

- Immunizations
- Transgenics
- Hybridoma
- Single B-cell
- Display systems for immune or naïve library generation
- Primary screening



### Lead Optimization

- Secondary Screening (Binding & Functional)
- Humanization
- Recombinant expression
- Manufacturability assessment
- Safety assessment
- Phenotypic biology



### Preclinical

- Bioanalytical testing
- PK/PD
- Immunogenicity
- *In vivo* efficacy
- Biomarker development



### Early Development

- Manufacturing support
- Cell banking
- Drug and product formulation
- QC testing and lot release



Eurofins Discovery Biotherapeutics Services



Eurofins DiscoverX Products



Eurofins BioPharma Services

# Summary & Conclusions

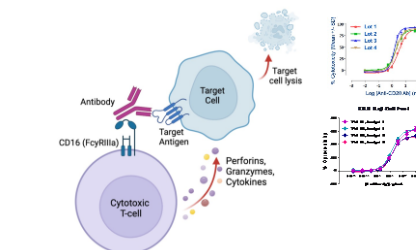
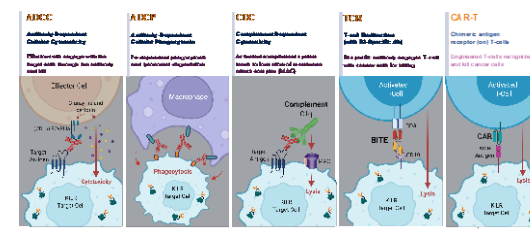
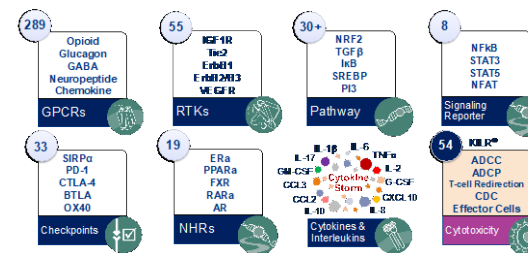
DiscoverX offers the industry's largest menu of cell-based assays with over 1500 off-the-shelf products

KILR<sup>®</sup> 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 CD16 Effector Cells offer a unique solution solving donor variability issue when sourcing effector cells

KILR Raji Bioassay allows discrimination of differences in Fab and Fc regions to enable rank ordering of antibodies



## Special Promotion

Pre-order your KILR Raji ADCC Bioassay Kits by June 30<sup>th</sup> & receive a **22% discount!**

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