HiBiT: A Tiny Tag to Assess MOA-based CAR-T Cell Potency

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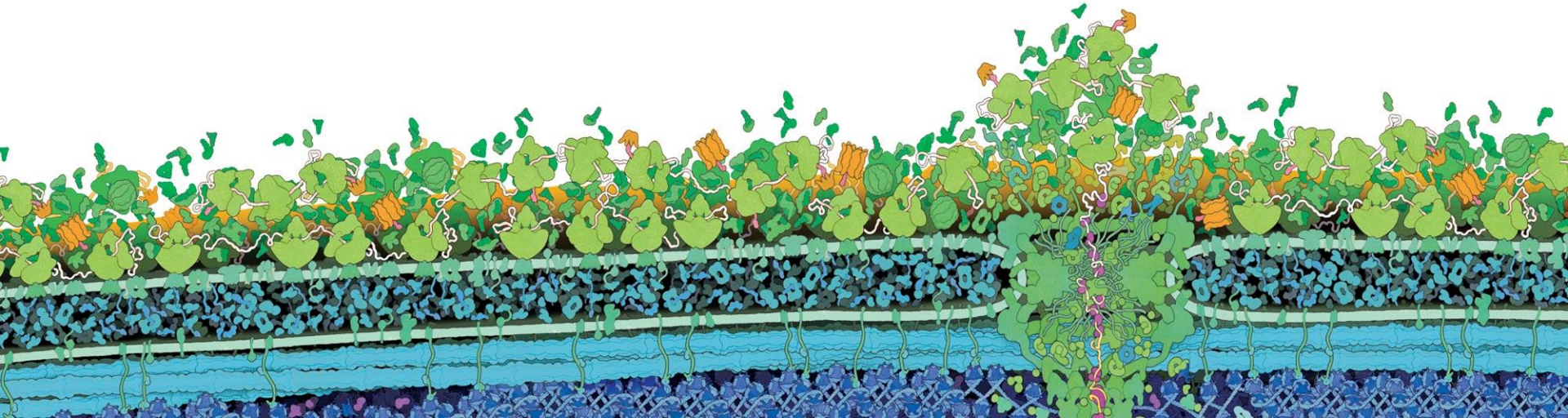
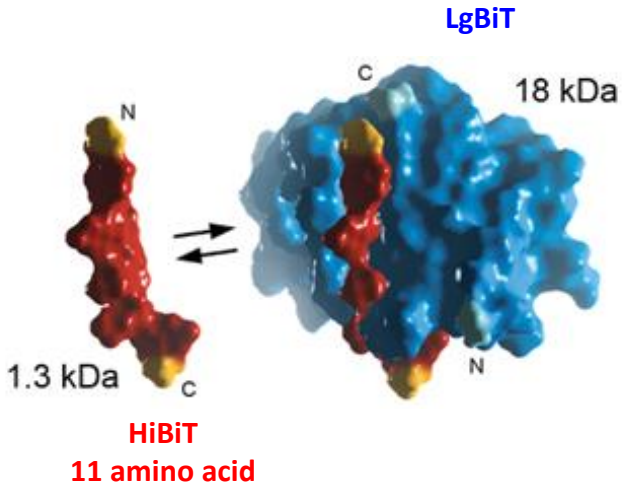
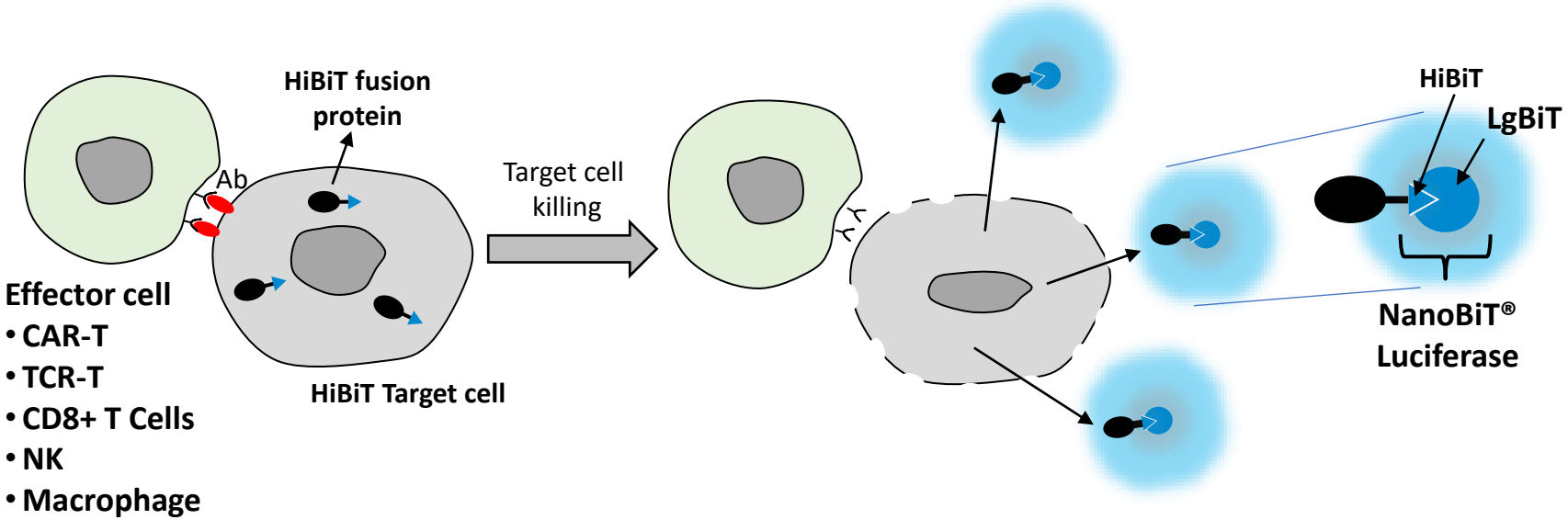


NanoBiT® Complementation Technology



- High affinity interaction between two subunits: HiBiT and LgBiT (K_D = 700 pM)
- Interaction forms functional NanoBiT luciferase and emits luminescence in the presence of substrate

HiBiT Target Cell Killing (TCK)
Assay Principle



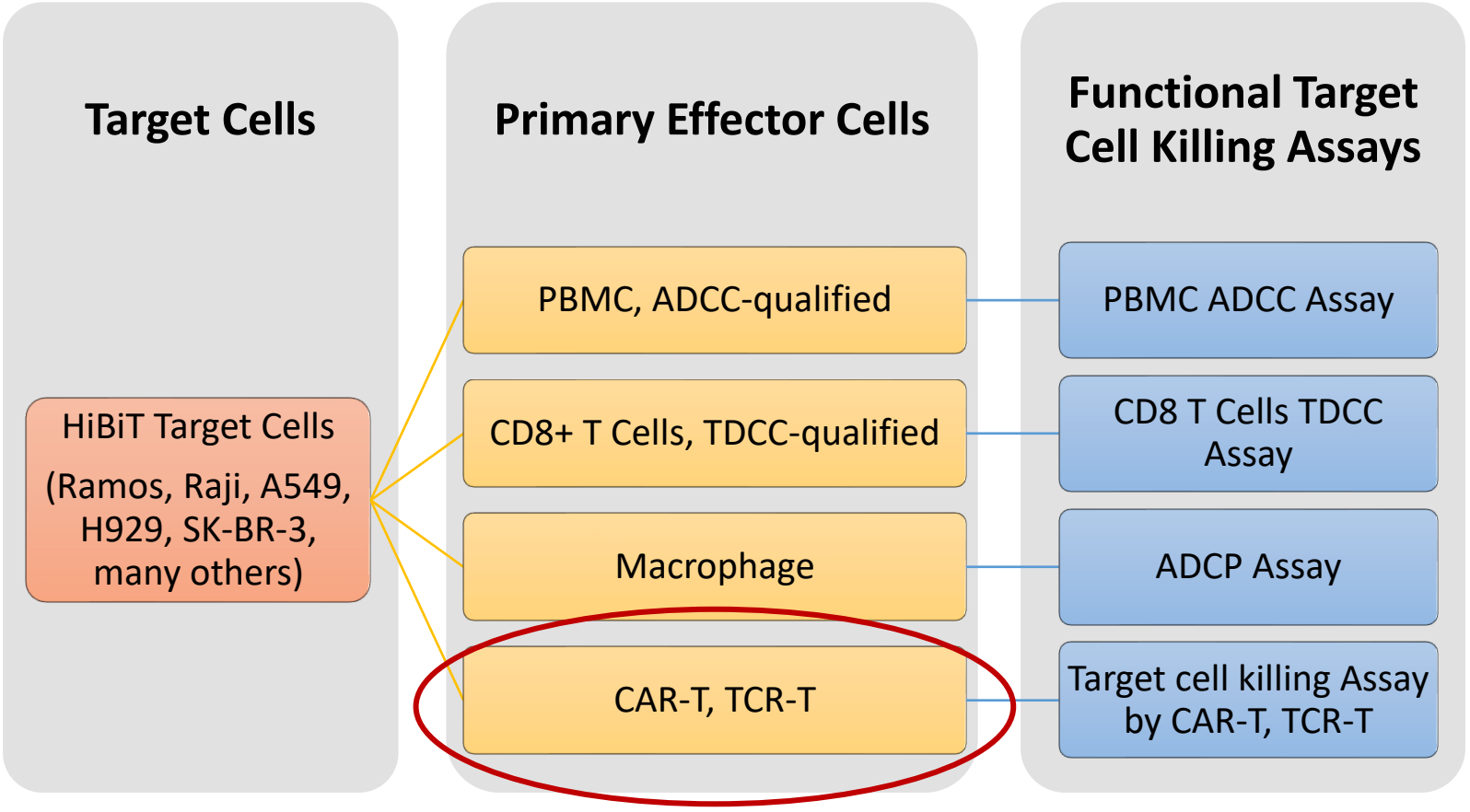
Assay Design

- Lysis of HiBiT target cells releases HiBiT into the medium
- HiBiT binds to LgBiT in the detection reagent, forms functional NanoBiT luciferase and emits luminescence

Features

- Measure target cell-specific killing
- Low spontaneous release
- Simple, homogenous
- No medium transfer required
- Measure assay response from hours to days

Versatile Target Cell Killing Platform

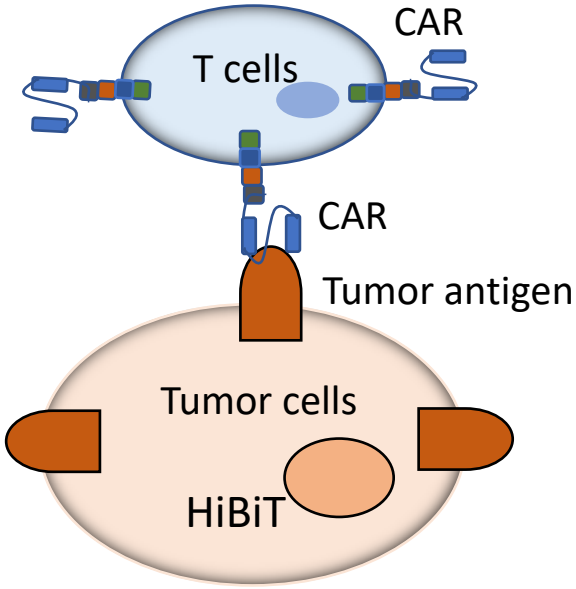


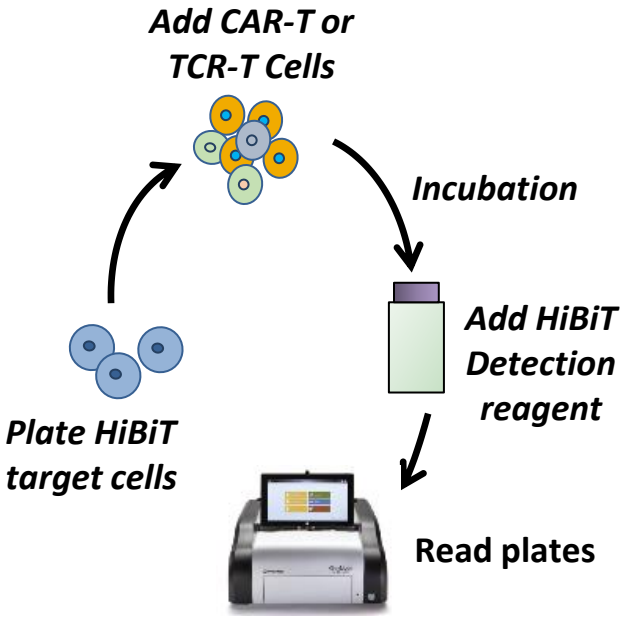
HiBiT Target Cells can be used to measure target cell killing by a variety of effector cells

Target Cell Killing Workflow

CAR-T and TCR-T Cell Therapies

Assay Design



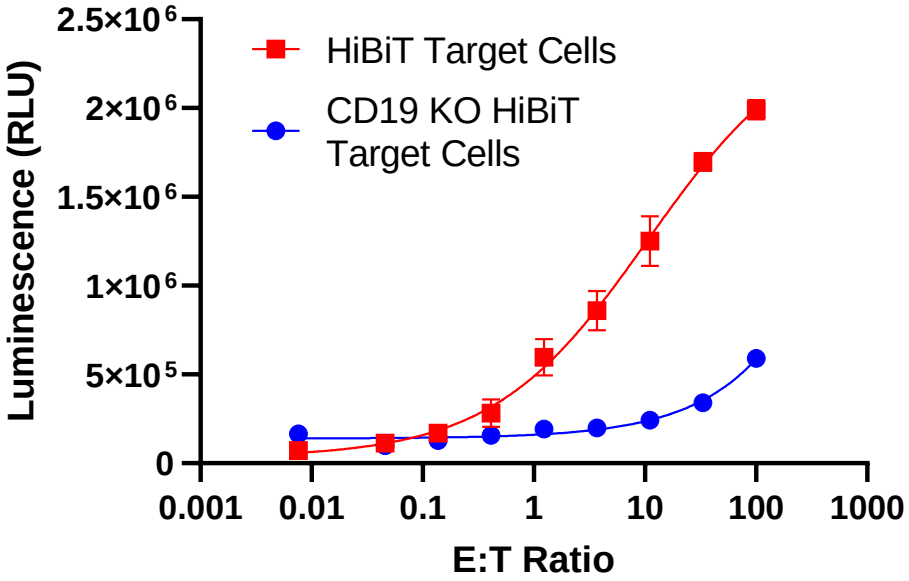


Assay procedure

1. Plate HiBiT target cells
2. Add CAR-T or TCR-T cells
3. Incubate for 4-72 hours
4. Add HiBiT detection reagent
5. Read plates

Assay Specificity

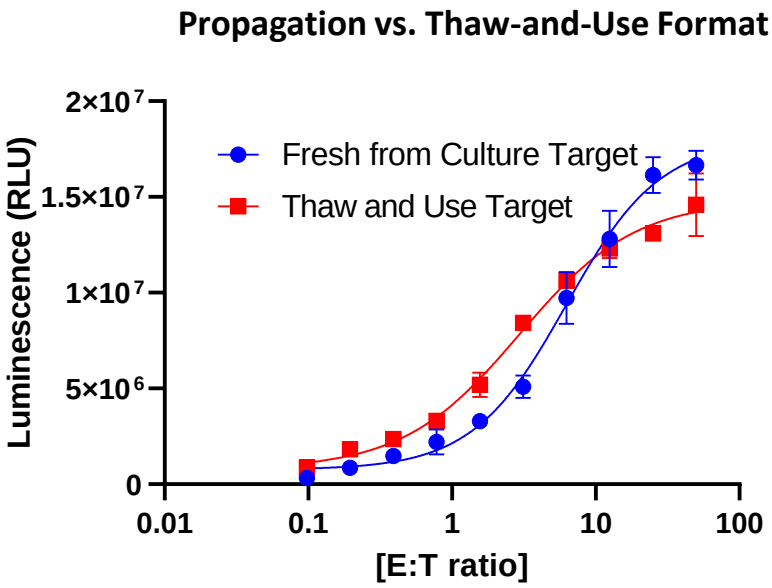
CRISPR-KO of Tumor Associated Antigens

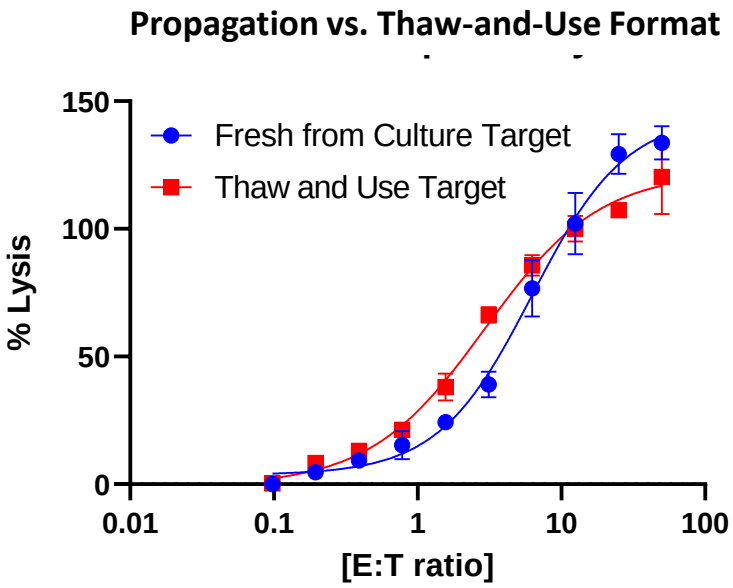


- CRISPR was used to knock out CD19 in Ramos HiBiT Target Cells
- CAR19 T Cells kill parental but not CD19-KO Ramos Target Cells

Assay Performance

CAR19 T Cell Killing of Ramos (HiBiT) Target Cells





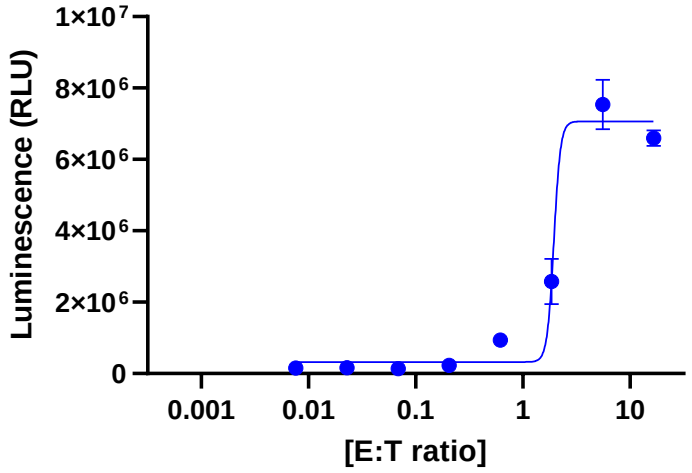
Similar results were achieved using HiBiT Target Cells in Propagation vs. Thaw-and-Use format

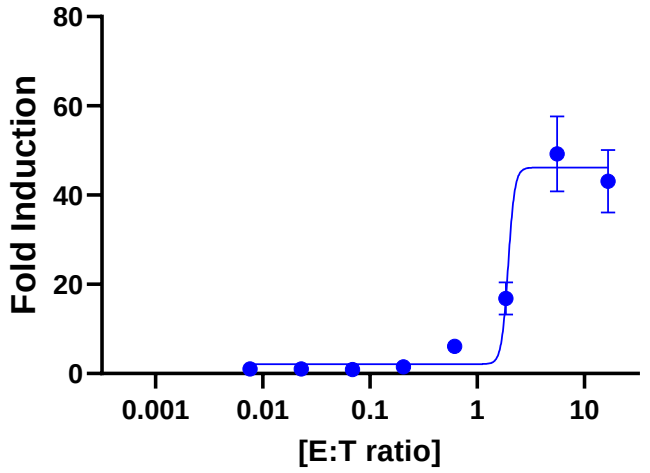
Assay Performance

CAR-BCMA T Cell Killing of H929 (HiBiT) Target Cells

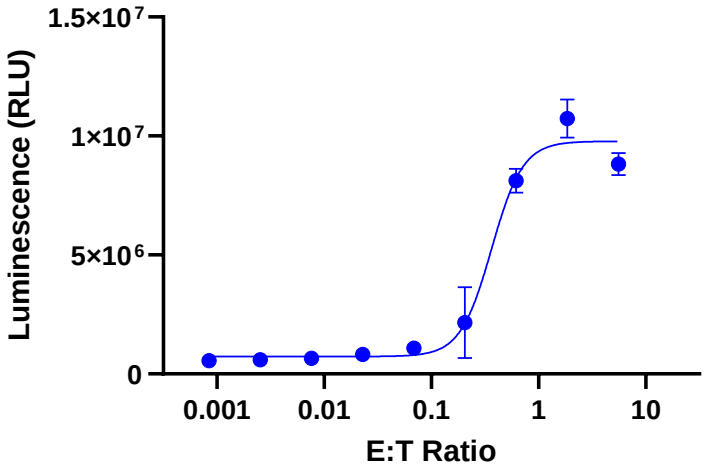
Luminescence | **Fold Induction**

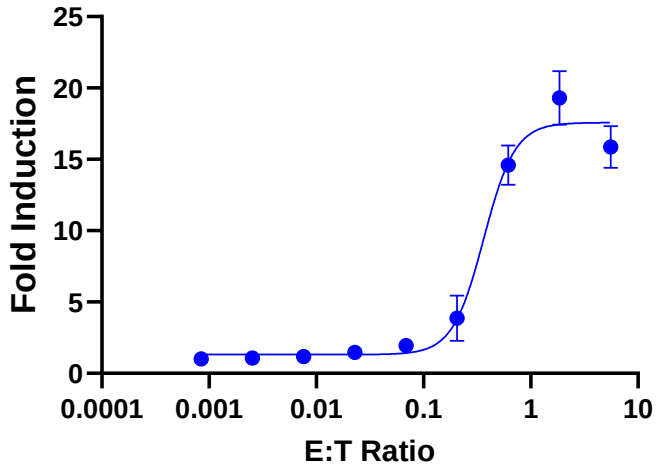
24 hrs





48 hrs





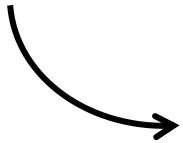
HiBiT Target Cell Portfolio

Endogenously Expressed Targets
Raji
Raji CD19-KO
Raji CD20-KO*
Raji CD19/CD20-KO*
Ramos
H929
A549
SK-BR-3
SKOV3
OVCAR3
OVCAR3 Mesothelin-KO*
U937
U937 CCL1-KO*
T2

Exogenously Expressed Targets
K562
K562+CD19
K562+BCMA
K562+GPC3*
K562+CIITA*
Membrane TNFα (CHO-K1)
SARS-CoV-2 S Protein (CHO-K1)

In Development

Don’t see what you need?



Biologics Assay Development & Services

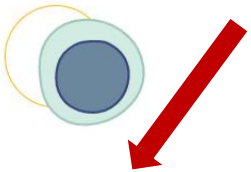
mAb Therapeutics

Potency Assay Development
- Genetic reporter bioassays
- PPI bioassays
- CRISPR engineering

Drug Profiling
- Genetic reporter bioassays
- PPI bioassays
- Primary cell assays

Bioassay Qualification
- Genetic reporter bioassays
- PPI bioassays

Made-to-Order Cell Manufacturing
- Master Cell Banks (MCB)
- Thaw-and-Use Cells



T Cell Therapy

TCR Discovery
- TCR functional assays
- Target cell killing assays
- CRISPR engineering

Potency Assay Development
- Target cell killing assays
- Cytokine bioassays

Drug Profiling
- Target cell killing assays

Bioassay Qualification
- Target cell killing assays

Made-to-Order Cell Manufacturing
- Master Cell Banks (MCB)
- Thaw-and-Use Cells

Gene Therapy

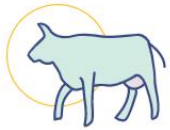
Potency Assay Development
- Genetic reporter bioassays
- PPI bioassays
- CRISPR engineering

Lumit™ Immunoassays
- Viral delivery (e.g. AAV)

Vaccines & Antiviral mAb

Assay Development
- Neutralizing Ab Function
- PsVLP bioassays
- Antibody Fc Function
- ADCC/ADCP target cells

Antibody Profiling
- ADCC/ADCP reporter bioassays
- PBMC ADCC bioassays
- Target cell killing assays
- Lumit™ Immunoassays



Veterinary/Animal Health

Vaccine Assay Development
- Neutralizing Ab Function
- Antibody Fc Function

mAb Assay Development
- Genetic reporter bioassays
- PPI bioassays
- Primary cell assays

Bioassay Qualification
- Genetic reporter bioassays
- PPI bioassays

Made-to-Order Cell Manufacturing
- Master Cell Banks (MCB)
- Thaw-and-Use Cells

Summary

The HiBiT Target Cell Killing bioassay platform uses HiBiT/LgBiT complementation technology to measure target cell-specific killing in mixed cell cultures with different effector cells

- Simple, fast, and sensitive assay platform to specifically measure killing of target cells
- Flexible platform to measure the activity of a variety of biologic drugs
- Off-the-shelf HiBiT Target Cells expressing common immunotherapy targets (e.g., CD19, CD20, BCMA, Mesothelin)
- Custom development of HiBiT Target Cells and assay optimization to meet your needs

The HiBiT Target Cell Killing bioassay platform can be used for potency testing and lot release of CAR-T cell products