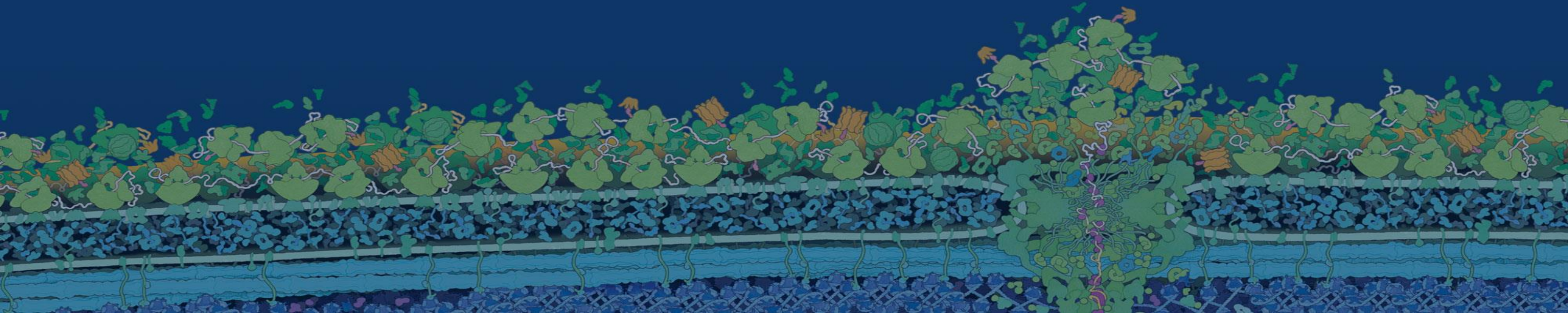
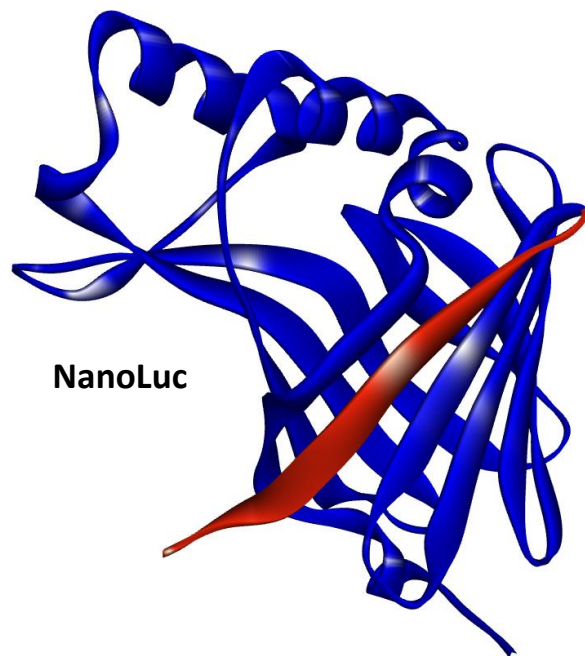


# Specific Detection of Target Cell Killing in Mixed Cultures Using HiBiT

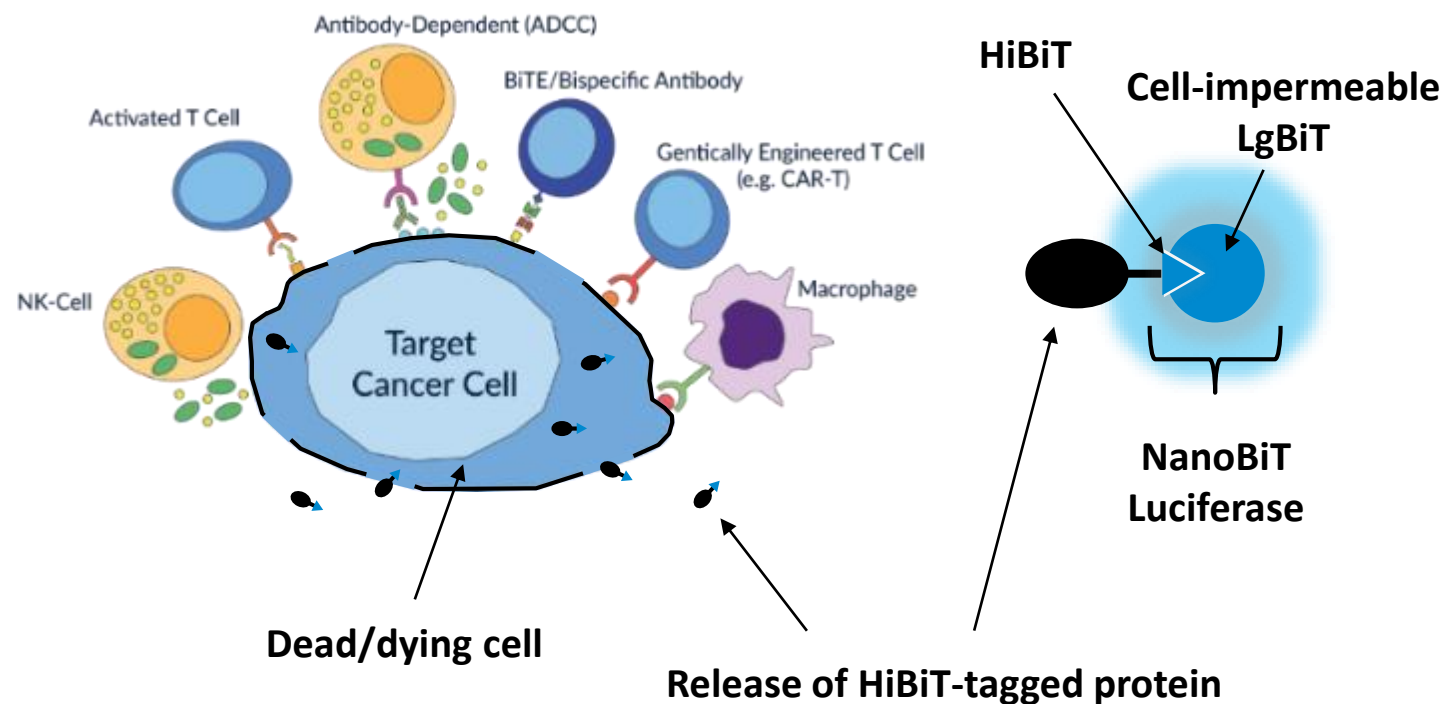


# HiBiT Target Cell Killing Assay (HiBiT TCK Assay)

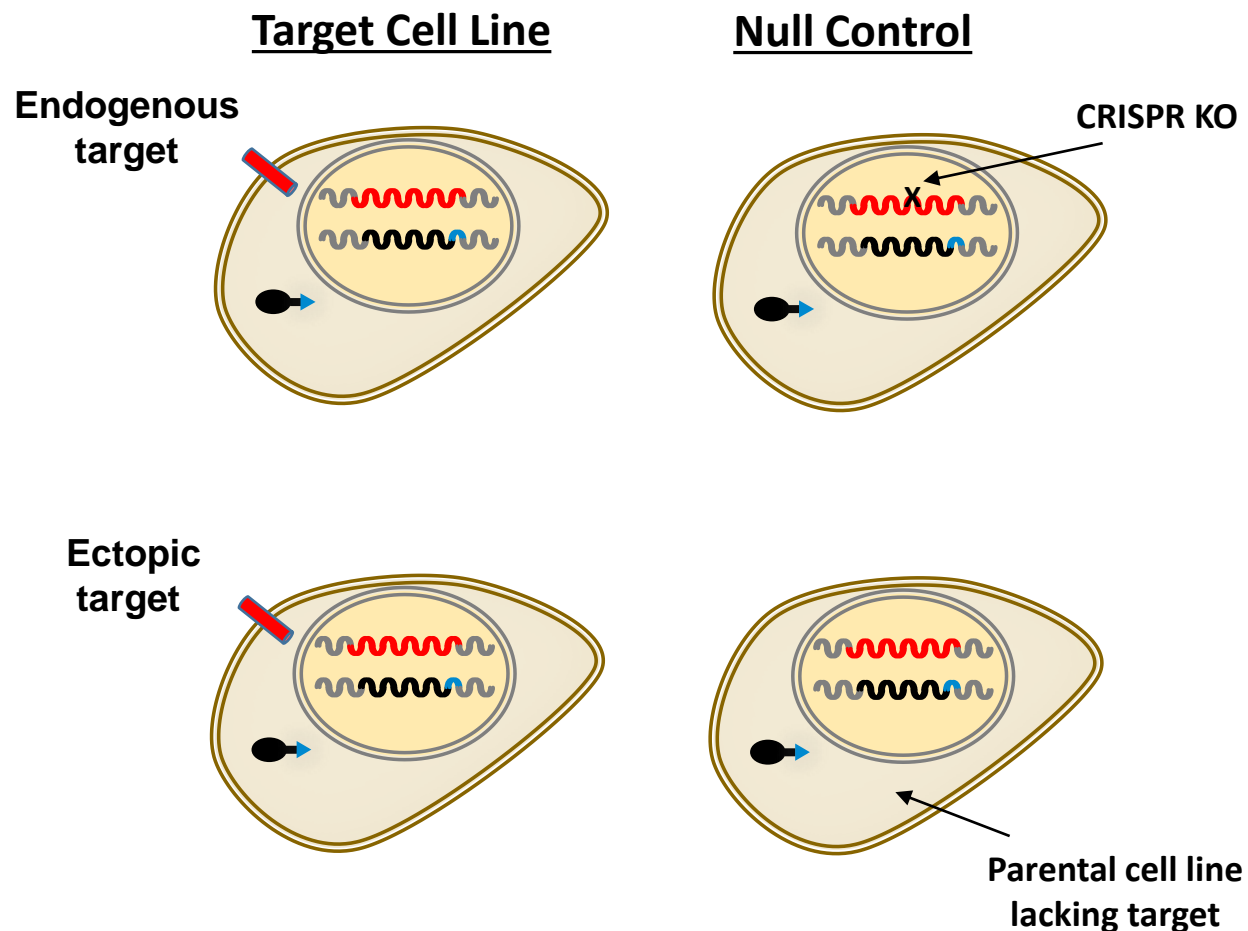
## HiBiT technology



- High BiT (HiBiT), 11 a.a. peptide
- Large BiT (LgBiT), 17.6 kDa
- High affinity interaction ( $K_D < 1$  nM)
- HiBiT:LgBiT = bright, luminescent enzyme



# Genetic Engineering of HiBiT TCK Cell Lines

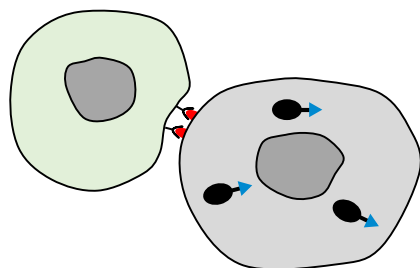


- Endogenous or ectopic expression of target
- Stable expression of HiBiT-tagged protein:
  - CRISPR knock-in (CRISPR KI) for endogenous expression of LDH-HiBiT
  - Random integration of plasmid DNA for ectopic expression of HaloTag-HiBiT
  - Lentiviral transduction for ectopic expression of HaloTag-HiBiT; lenti transfer vector available
- Null cell lines:
  - CRISPR/Cas9 homozygous knock-out for endogenous expression of target
  - Parental cell line for ectopic expression of target

# Endpoint or Kinetic Formats

## Endpoint Format

Target & effector cells



Add HiBiT Extracellular Reagent



Measure RLUs

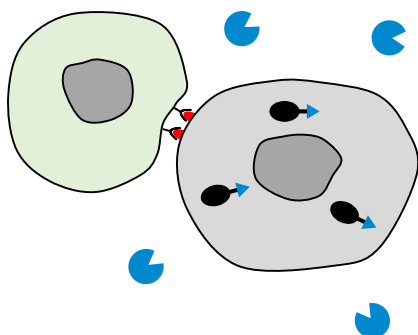


GloMax® Discover System

- Add-mix-read
- Measure TCK at single time point
- Add digitonin for Maximum Release control

## Kinetic Format

LgBiT & Endurazine added at  $t = 0$



Continuous measurements



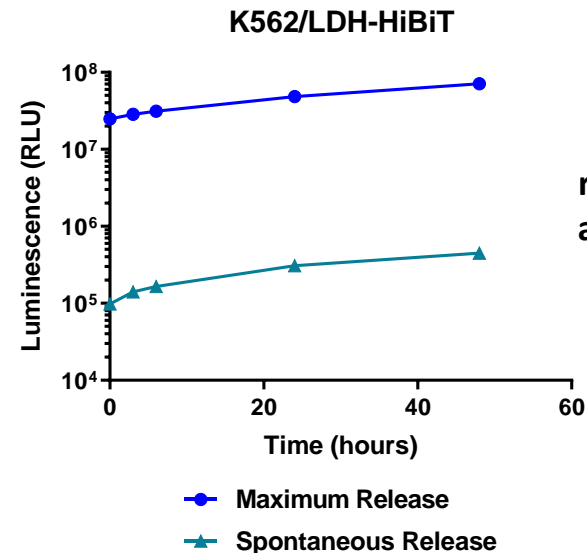
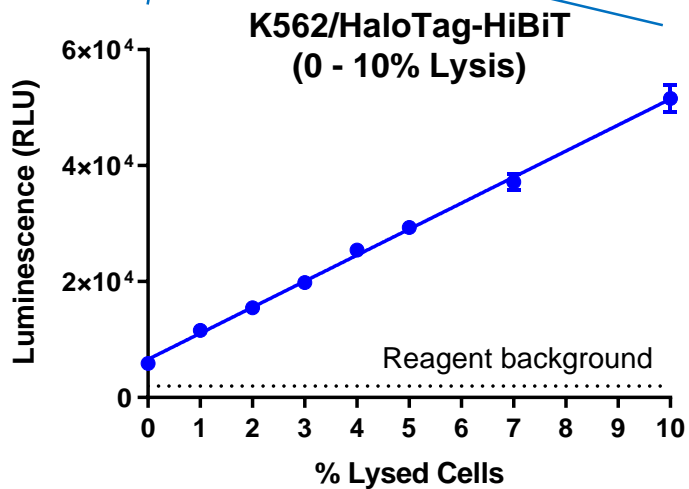
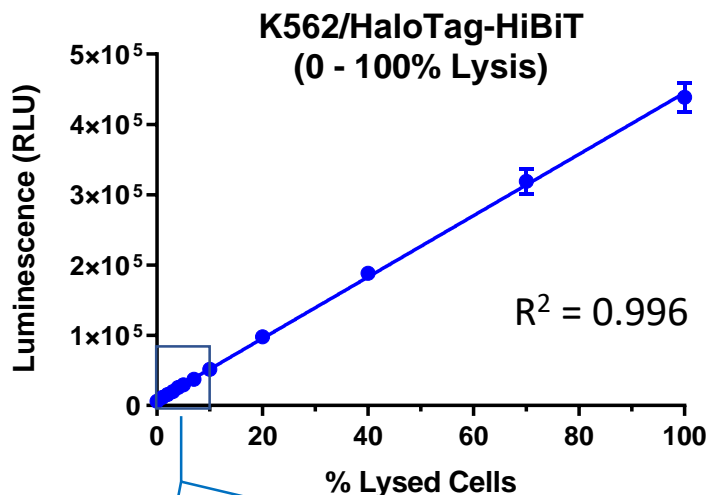
GloMax® Discover System

- Monitor TCK in real time
- Endurazine: modified furimazine for longer incubations

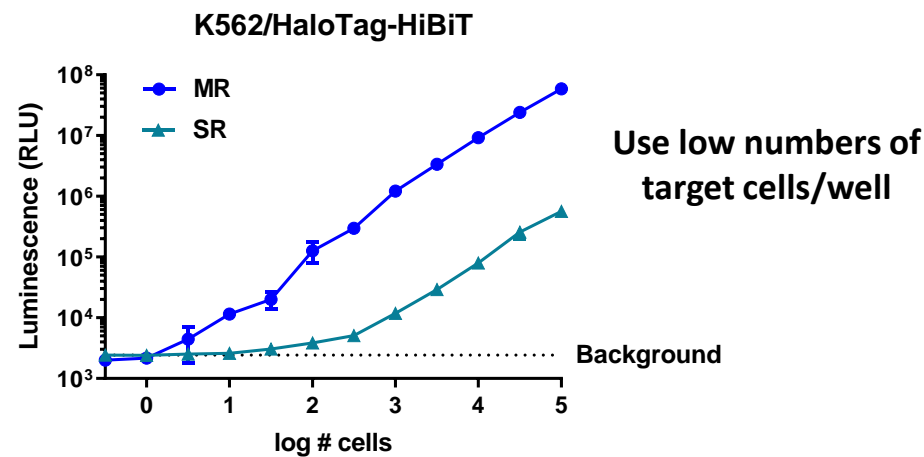


# Broad Linearity & High Sensitivity

- Mixture of sonicated plus healthy cells
- RLUs linear from 0-100% lysis
- Sensitive detection of low levels of cell lysis



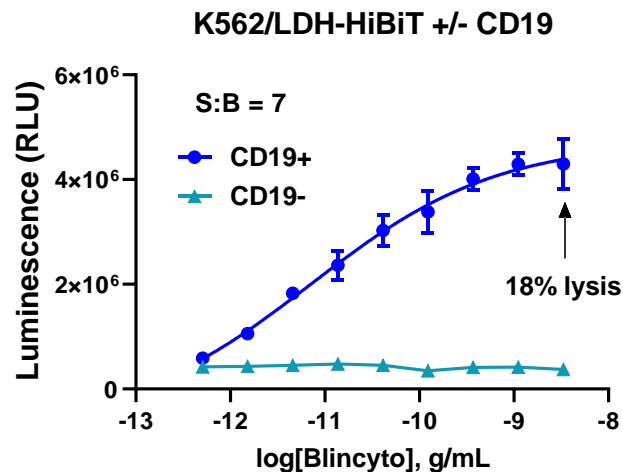
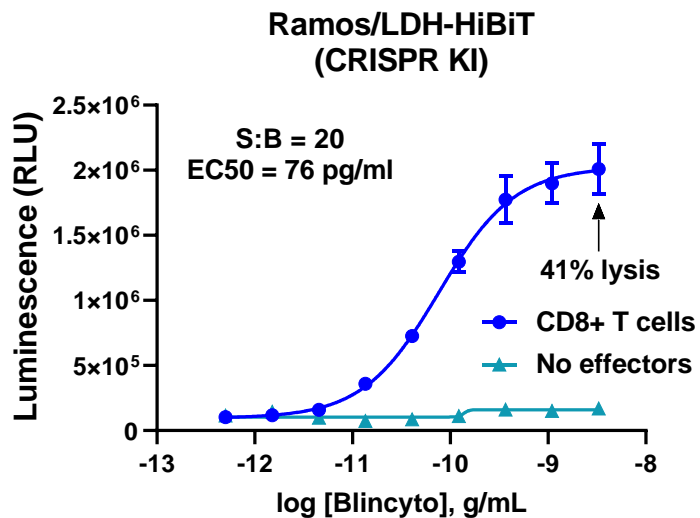
Low rates of spontaneous release & large assay windows



Use low numbers of target cells/well

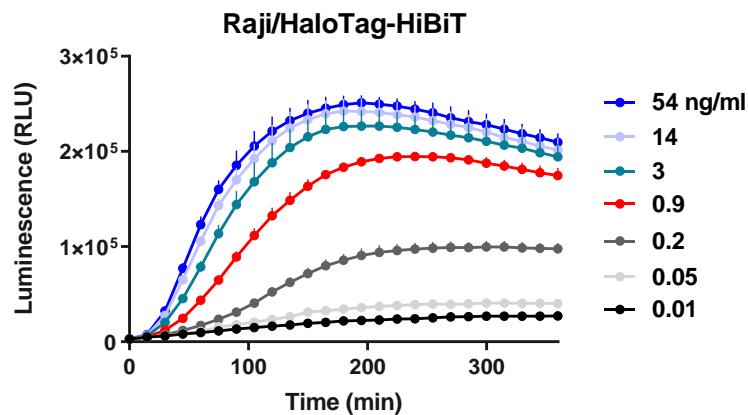
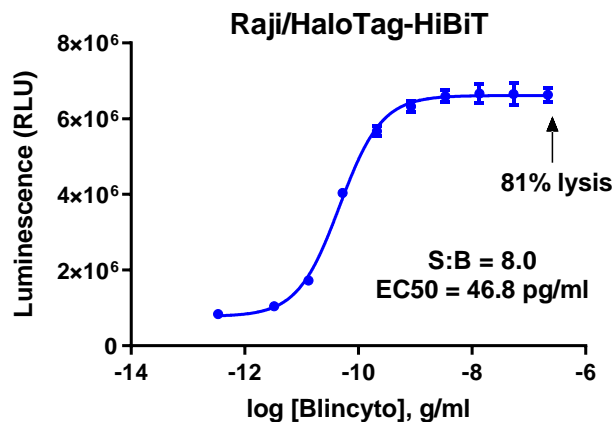
# Redirected T Cell Cytotoxicity Using Blincyto (Anti-CD19 BiTE)

Effectors:  
CD8+ T cells



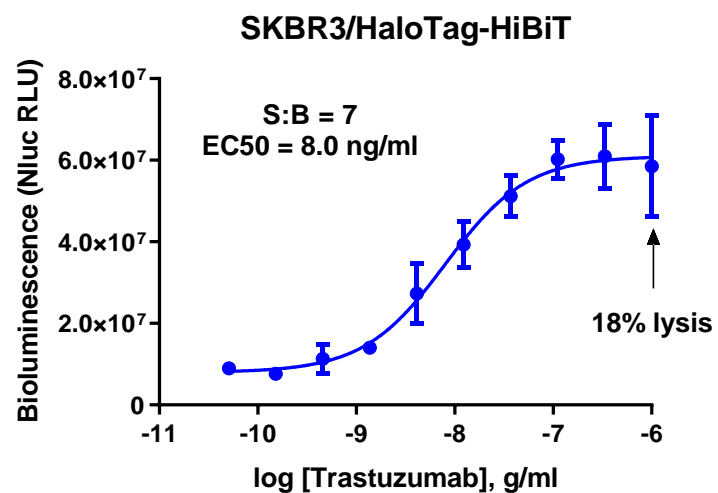
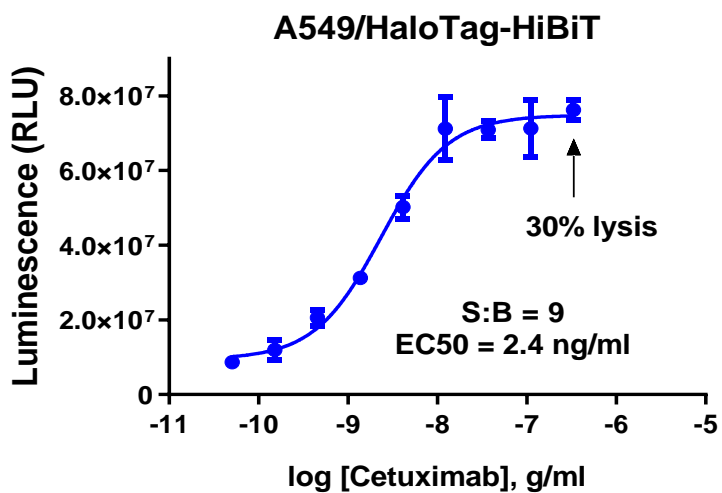
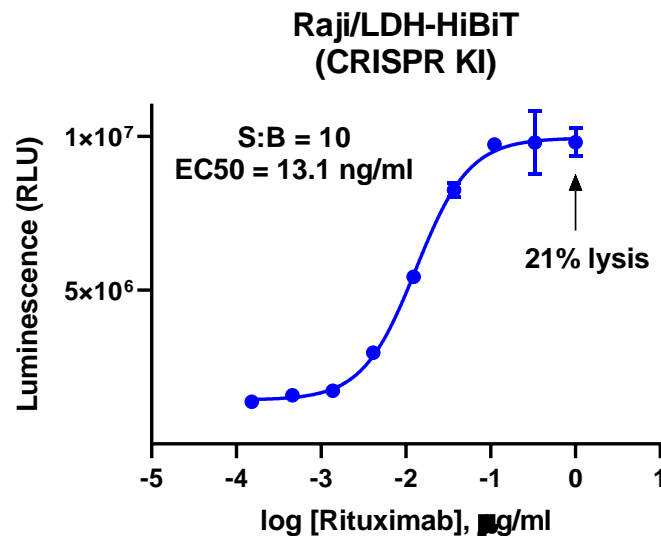
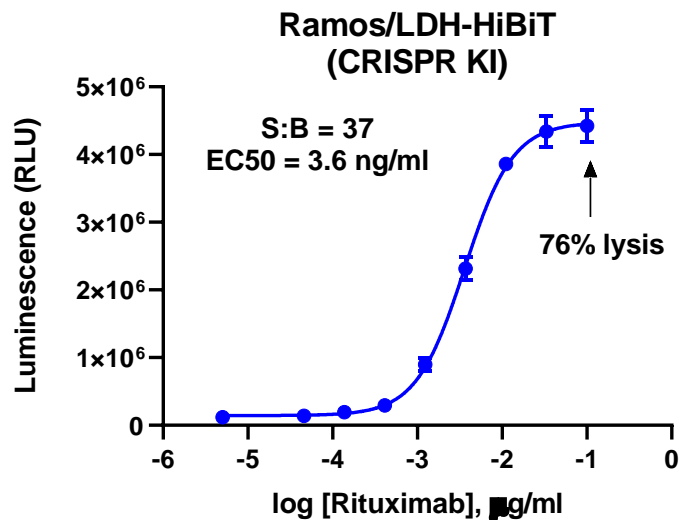
- 10:1 E:T ratio
- 2,500 target cells per well
- Overnight assays

Effectors:  
TALL-104 cells



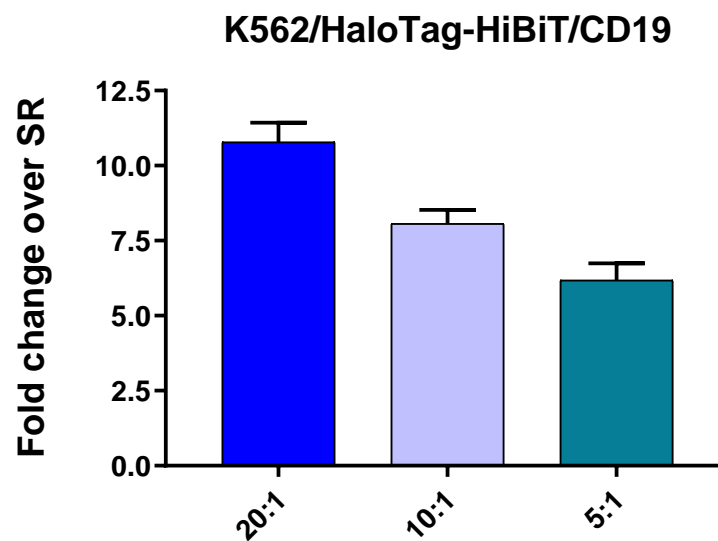
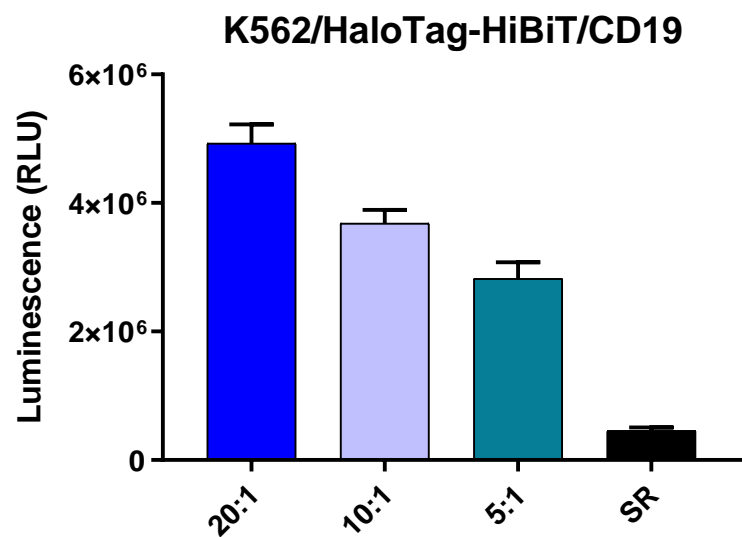
- 20:1 (left) or 5:1 (right) E:T ratios
- 10,000 target cells per well
- 6-hour endpoint assay (left)

# ADCC Assays Using PBMCs



- 20:1 E:T ratios
- 2,500 target cells per well
- 5-hour assays

# CAR-T Assays



- Varying E:T ratios
- 2,500 target cells per well
- 24-hour assay
- Anti-CD19 CAR-T cells from ProMab (PM-CAR1003)



# HiBiT TCK Cell Lines

## Available

Raji (CD19+, CD22+, CD20+)  
Ramos (CD19+, CD22+, CD20+)  
K562 (null background)  
K562/CD19  
K562/BCMA  
K562/MSLN  
U937 (CLL-1+, CD33+)  
A549 (EGFR+, EphA2+)  
SKBR3 (HER2+)

## Under construction

Raji (CD19 KO)  
Ramos (CD19 KO)  
SKOV3 (MSLN+, 5T4+, MUC16+, HER2+)  
H929 (BCMA+)  
OVCAR3 (MSLN+, 5T4+, WT1+, HER2+)

- **Cell lines can be made as custom projects**
- **Protocols/reagents for DIY are available**

**Cell line list as of 10/17/19**

**For an updated list, contact [Maggie.Bach@Promega.com](mailto:Maggie.Bach@Promega.com)**



## Summary

**Selectively monitor the death of target cells in mixed cultures:** Compatible with ADCC, RTCC & CAR-T/TCR-T assay formats

**Sensitive assay for Target Cell Killing (TCK):** Detect low levels of TCK in mixed cultures; compatible with a wide range of target cells per well, including low numbers.

**Endpoint assay:** Add detection reagent at single time point.

**Kinetic assay:** Add reagents to cell culture medium at the start of the assay, continuously read luminescence for 24 hours or more.

**Low rates of spontaneous release:** Run assays for  $\geq 24$  hours

**Compatible with thaw-and-use format:** Thaw target cells and use the same day.