A Homogeneous Bioluminescence Cell-Specific Cytotoxicity Assay

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1. Introduction

As our understanding of disease evolves so does the need for developing innovative tools to discover novel therapies using high-throughput screening (HTS). Cell-cell interactions within co-culture systems have increasingly been recognized to better model human biology for identification of putative drug targets. Historically, bioluminescence viability and cell death assays have long been used in HTS owning to its high sensitivity, scalability, and signal robustness. While these assays are an excellent means for quantifying cell health in bulk formats the hurdle of measuring death of a specific sub-population of cells in a co-culture system remained. To address this unmet need, we present a bioluminescent add and read assay that can readily detect cell-specific death of any cell type of interest, including primary cells. The assay principal is based on rapid covalent labeling of intracellular lysine residues using a chloroalkane ligand in living cells. Upon cell lysis, the label is released into cell medium and can be quantified using bioluminescence detection reagents. Utility of this system is demonstrated through antibody dependent cell-mediated cytotoxicity (ADCC) and CAR-T target cytotoxicity assays. Cell death can be measured kinetically or as endpoint measurements in a variety of assay plate formats. Together, we demonstrate a simple, scalable and flexible, non-radioactive add and read system for measuring cell specific cytotoxicity within a mixed population.

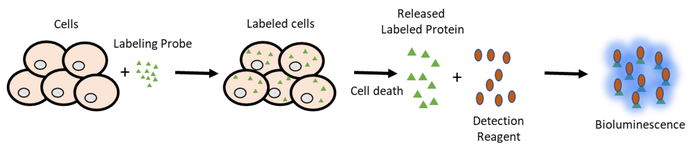
2. Assay Overview

Assay Principle

A bioluminescent detection system to measure death of a specific cell population within a mixed population of cells without cellular engineering.

Assay Concept

1. Covalently label intracellular proteins (NH2 groups) of target cells.
2. When cells undergo cell death the cell membrane deteriorates and labeled proteins are released into culture media.
3. The amount of labeled proteins released into media is a direct indicator of cell death
4. Labeled proteins are quantified using a bioluminescence detection system.



Schematic of labeling and detection of released labeled proteins. Cells are covalently labeled using a small molecule labeling probe. Upon cell death labeled proteins are released and detected using bioluminescence detection reagents. Light generated is proportional to incidence of cell death.

3. Primary Applications

1. Cell-mediated cytotoxicity (CMC) - the death of target cells induced by effector cells
2. Antibody-dependent cell-mediated cytotoxicity (ADCC)
3. Specific cell-death in co-cultures (3D-organoids, tumor environment), applicable to primary cells

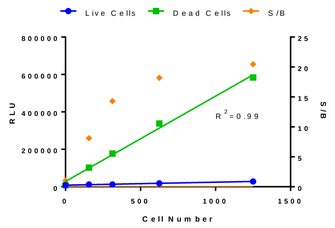
4. Applicable to Any Cell Type with LOD <100 cells

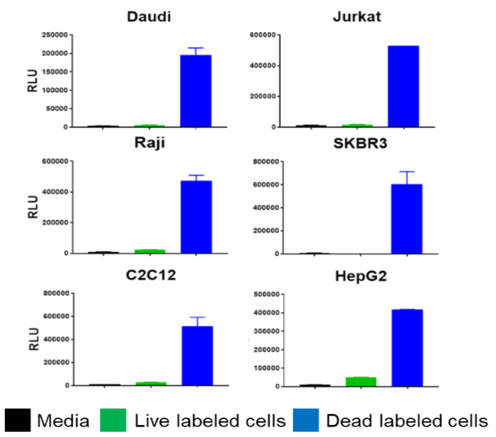
Cell Labeling Protocol

Collect and spin desired cell type
↓ 5 min at 300g
Aspirate media and re-suspend in PBS
↓
Add labeling probe
↓ 30 min at 37°C
Add pre-warmed (37°C) complete media containing 10% serum
↓ 5 min at 37°C
Spin labeled cells and remove excess media
↓ 5 min at 300g
Re-suspend cells in desired media
↓
Set up cells for experiment

Cell Death Detection

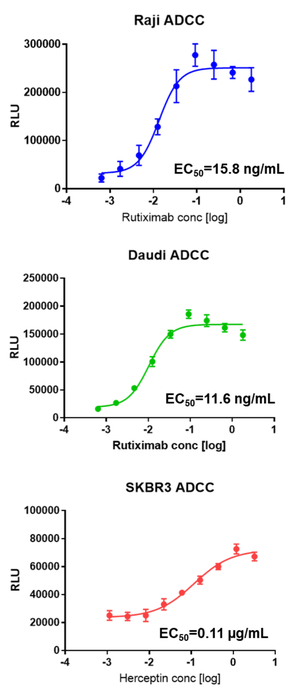
Add detection reagent
↓ 30 min at 37°C
Measure bioluminescence



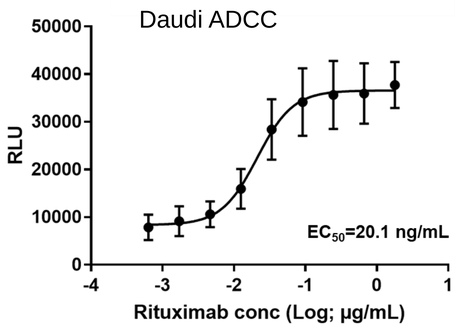


5. Antibody Dependent Cell-Mediated Cytotoxicity (ADCC) in Multiple Cell Types

Prepare Effector cells (PBMC)
↓
Label target cells
↓
Mix PBMCs and labeled target cells
↓
Plate cells in media
↓
Add Antibody
↓
Incubate
↓
Add detection reagents
↓
Measure



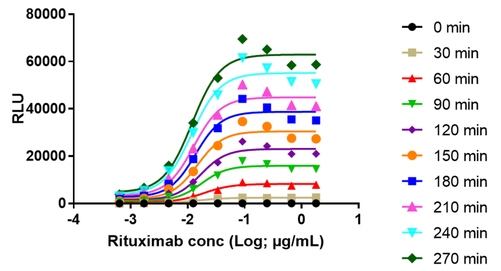
6. ADCC Scaled to 384-well Low Volume Format



7. ADCC Measurements in Real-Time

Prepare Effector cells (PBMC)
↓
Label target cells
↓
Mix PBMCs, target cells, Ab, and detection reagents
↓
Incubate
↓
Measure over time

Nano-Glo Vivazine Live Cell Substrate



minutes	0	30	60	90	120	150	180	210	240	270
EC50	NA	0.01504	0.01908	0.01767	0.01698	0.01483	0.01373	0.01277	0.01187	0.01191

8. Conclusions

We present a novel homogeneous bioluminescent-based target cell killing assay that readily detects death of a any cell type within a mixed population

No radioactivity

Rapid labeling of any cell type of interest, including primary cells

Covalent labeling coupled with bioluminescent detection reagents
- Reduces 'leakiness' over assay duration
- Increases sensitivity and specificity (LOD<100 cells)

Experimental flexibility
- Homogeneous
- Endpoint or kinetic measurements.

Scalability
- ADCC in 384-well low volume formats

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