A Versatile Platform for Cell Therapy Cytotoxicity Assays Using mRNA Transfection

Brock Binkowski, Julia Gilden, Nicholas Hess, Aileen Paguio, Mei Cong & Marjeta Urh

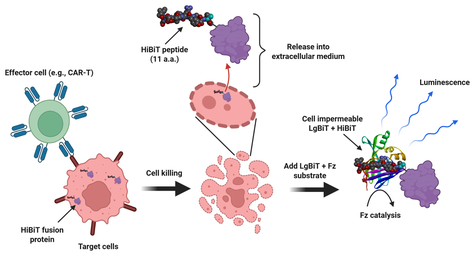
Promega Corporation, 2800 Woods Hollow Rd, Madison WI, 53711

1. Introduction

The HiBiT Target Cell Killing Bioassay (HiBiT TCK Bioassay) is a sensitive and selective method for quantifying target cell lysis in coculture experiments with effector cells. In this assay, target cells engineered to express a HiBiT-tagged protein are cocultured with effector cells, where target cell death promotes release of the HiBiT-tagged protein into the extracellular medium. Once there, HiBiT can bind to cell-impermeable LgBiT to generate a bright, luminescent enzyme in the presence of furimazine substrate. In this manner, a non-lytic detection reagent containing LgBiT and furimazine substrate can be used to quantify target cell death in a gain-of-signal assay format with a cumulative readout. To date, this approach has been used exclusively with stable expression of the HiBiT-tagged protein, requiring time and effort to make stable clones.

To expand beyond the use of stable clones, we developed a novel mRNA transfection reagent that enables rapid and uniform expression of HaloTag-HiBiT in a wide range of target cell types. This approach dramatically reduces the workload for creating HiBiT target cells, allowing users to readily label target cell type(s) of interest. We demonstrate labeling of a variety of target cell types and their use in the HiBiT TCK Bioassay. Additionally, we demonstrate the use of mRNA transfection to achieve uniform and highly tunable expression of target receptors in cells stably expressing a HiBiT-tagged protein, allowing drug potency/efficacy testing across a broad range of receptor expression levels in a single cell type.

2. HiBiT Target Cell Killing (TCK) Bioassay

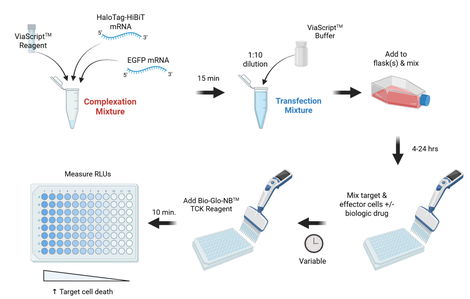


- TCK leads to release of HiBiT-tagged protein from target cells
- **Signal from target cells alone**
- HaloTag-HiBiT stable in extracellular medium (cumulative read of TCK)

For a complete listing of available target cell lines, including target receptor KOs, visit: https://www.promega.com/products/reporter-bioassays/target-cell-killing-bioassays/

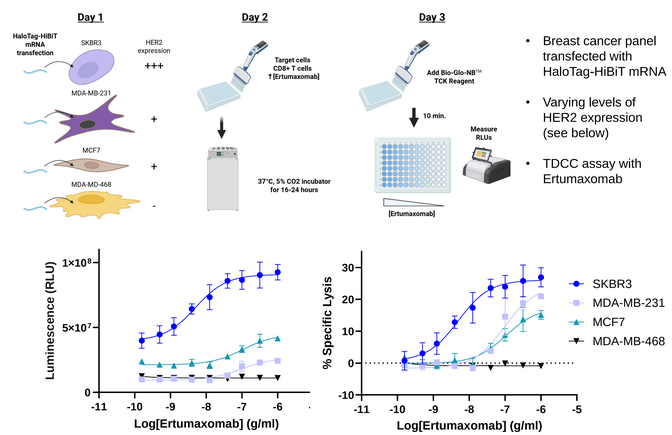
3. ViaScript™ TCK Bioassay

Extending the HiBiT TCK Bioassay beyond use of stable clones



- Efficient, low-toxicity transfection across diverse cell lines (adherent and suspension) using a novel mRNA transfection reagent
- 5MoU modified mRNA for increased intracellular stability; HaloTag-HiBiT expression is maintained for days
- mRNA transfection using ViaScript™ provides uniform & highly titratable expression (unlike plasmid DNA transfection)

4. Easily Screen Panels of Target Cells

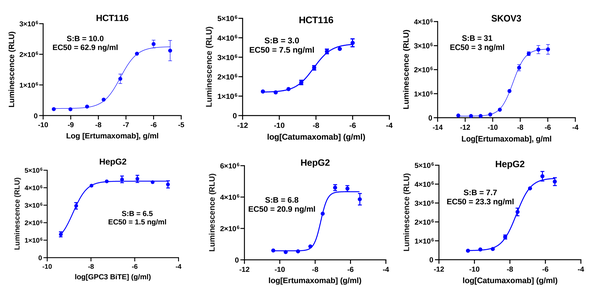


- Breast cancer panel transfected with HaloTag-HiBiT mRNA
- Varying levels of HER2 expression (see below)
- TDCC assay with Ertumaxomab

- **Compare potency & efficacy using panels of disease-relevant cell lines**
- Pick a cell background for stable expression

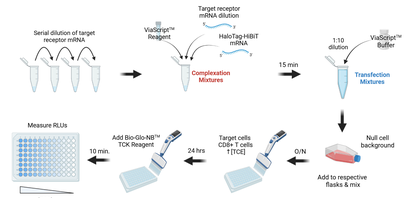
ViaScript TCK Approach Applied to a Breast Cancer Panel. Day 1, transfection of HaloTag-HiBiT mRNA using ViaScript™ Reagent. Day 2, mixing of CD8+ T cells with target cells (10:1 E:T ratio) in the presence of varying concentrations of Ertumaxomab (HER2 X CD3). Day 3, addition of Bio-Glo-NB™ TCK Reagent followed by luminescence measurement.

6. Further POC with Adherent Cells

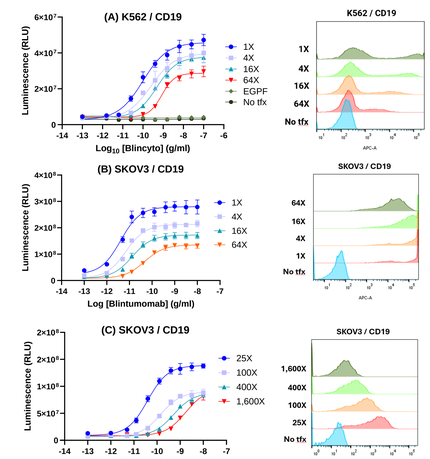


TDCC with Adherent Cells. Day 1, transfection of HaloTag-HiBiT mRNA using ViaScript™. Day 2, mixing of CD8+ T cells with target cells (10:1 E:T ratio) in the presence of varying concentrations of T cell engagers. Day 3, addition of Bio-Glo-NB™ TCK Reagent followed by luminescence measurement.

7. Titrate Target Receptor Expression in the Same Cell Background



- Serial dilution of target receptor mRNA (1X, 4X, 16, 64X, etc.)
- Transfect into null cell background



- **Compare potency & efficacy at varying levels of target expression using the same cell background**
- More uniform and titratable expression of target receptor than plasmid DNA transfection
- Compatible with a broad range of cell types (adherent & suspension)
- Co-transfect HaloTag-HiBiT mRNA to selectively measure death of target cells using HiBiT TCK Bioassay

Modulating CD19 Expression in K562 & SKOV3. Day 1, CD19 mRNA transfection using ViaScript™ of a K562 (HaloTag-HiBiT) stable cell line or parental SKOV3 (also transfected with HaloTag-HiBiT). Day 2, mixing of CD8+ T cells with target cells (10:1 E:T ratio) in the presence of varying concentrations of Blincyto (CD19 X CD3). Day 3, addition of Bio-Glo-NB™ TCK Reagent followed by luminescence measurement. Panels A, B & C are independent experiments.

8. Summary

- **HiBiT TCK Bioassay:** Release of HiBiT-tagged protein from dead/dying cells leads to signal increase; no signal contribution from effector cells
- **ViaScript™ TCK Bioassay:**
  - Transfection of HaloTag-HiBiT mRNA using a novel reagent
  - Easily apply to panels of disease-relevant cell lines
- **Modulate target receptor expression in the same cell background:**
  - Highly titratable (far better than use of plasmid DNA)
  - Determine drug product potency & efficacy as a function of target receptor molecules per cell
  - HiBiT TCK Bioassay readout when co-transfecting HaloTag-HiBiT mRNA
- Available for purchase via Promega's Early Access Program. Contact nicholas.hess@promega.com.

Corresponding author: brock.binkowski@promega.com