New Sensitive Luminescent LDH-release Assay that Enables “Real-Time” Sampling from Individual 3D Microtissues

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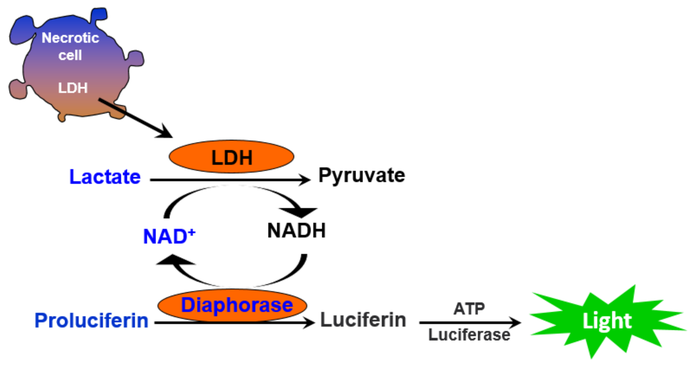
Abstract # SLAS-1183 / P030

1. Introduction

We developed an improved LDH-release assay that detects fewer than 10 dead cells. Small aliquots of culture medium can be removed at various times from the same sample of cells and assayed for LDH activity to monitor cell death over time. We will show examples monitoring LDH-release over time from individual ~250µm diameter 3D liver spheroids. We will present results from individual 3D tumor spheroids showing multiplex detection of LDH to measure dead cells and a real-time viability assay to estimate live cells. We also will show examples using LDH-release for the detection of cytotoxicity of target cells resulting from treatment with an antibody drug conjugate or an antibody dependent cell-mediated cytotoxicity approach using a mixture of effector and target cells. This new sensitive method for measuring LDH enables multiple sampling of culture supernatant from the same cells to enable real-time monitoring of cell death from individual 3D microtissues and mixed cell culture models.

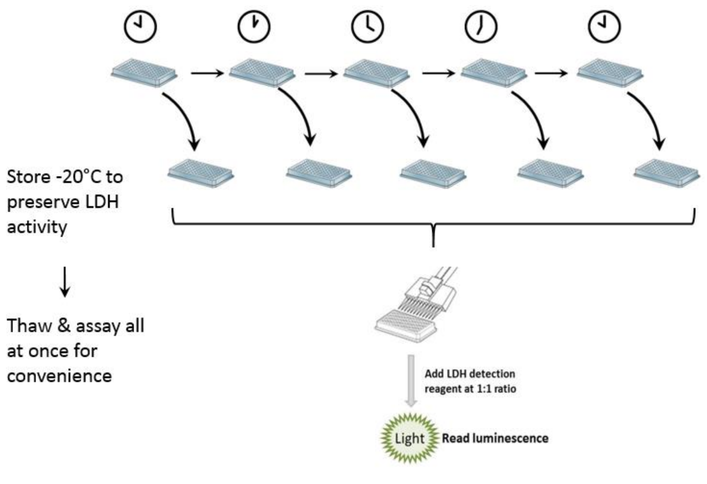
2. Schematic and Workflow Diagram for Luminescent LDH-release Assay



3. Sampling Culture Medium at Different Times can Mimic a “Real-Time” Approach

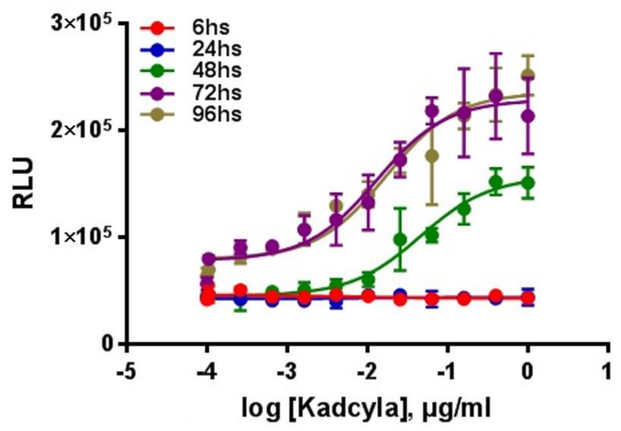
Sampling culture medium at time intervals and storing samples frozen avoids stability problems for LDH in culture medium.

Samples can be thawed and assayed all at once.



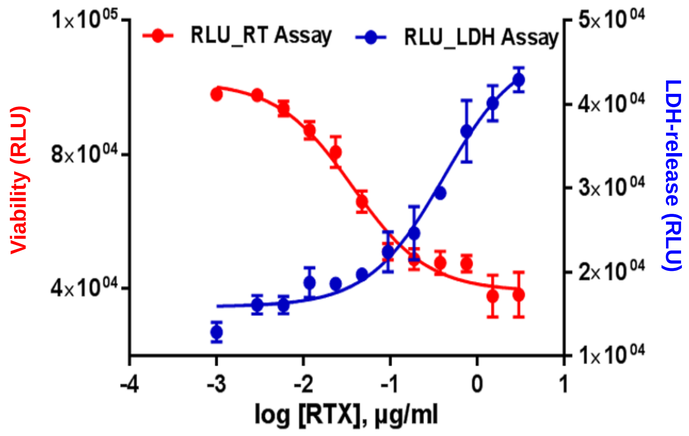
4. LDH-release (Cell Death) Time Course After Treatment with Antibody-Drug Conjugate

- Kadcyla was added to HER2 positive SKBR3 cells
- 4µl aliquots of culture medium were removed at 6, 24, 48, 72 & 96 hours, diluted with 96µl of Storage Buffer and stored frozen
- Samples were thawed and LDH measured by combining 9µl Sample + 9µl LDH Reagent in white 384 well plate



5. Multiplex Detection of ADCC Using “Real-Time” LDH-release and Viability Assays

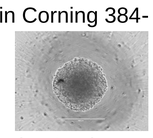
The LDH-Glo™ Cytotoxicity Assay and the RealTime-Glo™ MT Cell Viability Assay were multiplexed as orthogonal methods to detect dead cells and viable cells from the same samples.



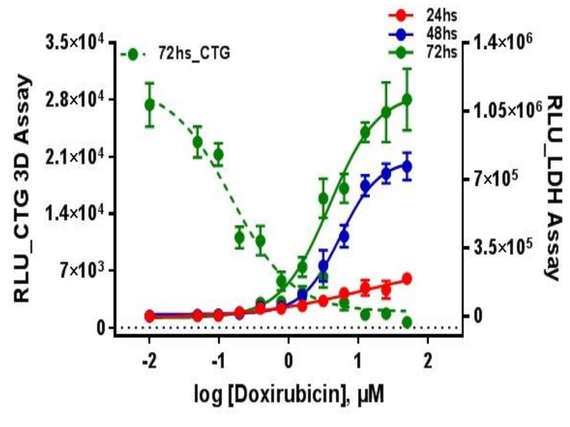
Cells were plated at 20:1 Effector (PBMC):Target (Daudi) ratio and treated with serially diluted rituximab for 4 hours. Medium samples were removed to measure LDH as a marker for cytotoxicity. Then the RealTime-Glo™ Reagent was added to the cell samples and luminescence measured to determine cell viability.

6. Multiplexing LDH-release (Cell Death) and ATP (Viable Cell Number) Assays

- HCT116 spheroids were formed using 2,500 cells/well in Corning 384-well Ultra-Low Attachment plates (scale bar = 400 µm)

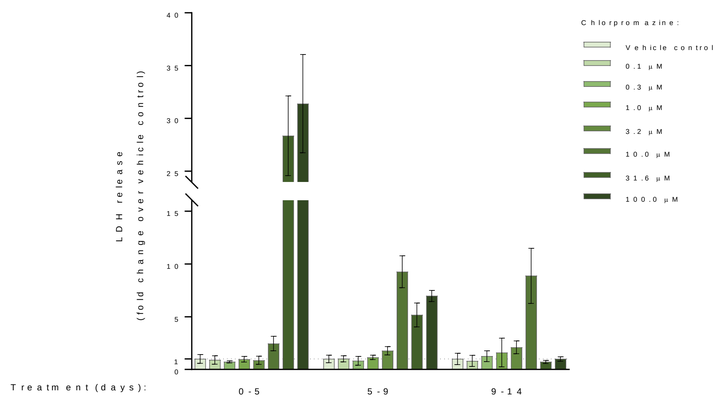


- Changes in toxicity during treatment were determined using luminescent LDH-release assay
- At the end of the treatment CellTiter-Glo® 3D Viability Reagent was added to the same wells determine ATP as a marker of viable cells

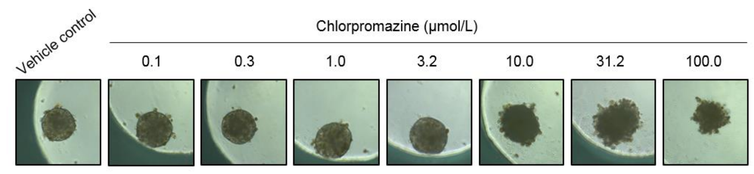


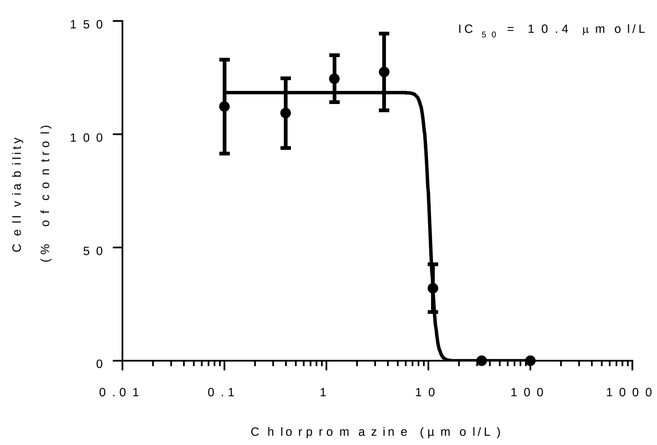
7. LDH-release from 3D InSight™ Human Liver Microtissues Exposed to Chlorpormazine

3D InSight™ Human Liver Microtissues (co-culture of primary hepatocytes, Kupffer cells and liver endothelial cells) were exposed to Chlorpormazine for 14 days (Dosing at day 0, 5 and 9). Time- and dose-dependent cytotoxicity was assessed by LDH-release assay. Released LDH was measured in supernatants at each re-dosing and expressed as fold change over vehicle control (mean of n=4 with SD. LDH-Glo Assay allows for identification of necrotic hepatocellular injury and assesment of its kinetics, and can be multiplexed with microtissue lytic endpoints.



Bright field images of microtissues from each treatment group at the end of treatment. Change of microtissue appearence due to necrosis observed at concentration 10 µmol/L and higher.





ATP assay for cell viablilty (CellTiter-Glo® 2.0 Cell Viability Assay) was done at end of treatment period (at 14 days) as an orthogonal method to demonstrate cytotoxicity (mean of n=4 with SD).

8. Summary

LDH leakage from dead cells into culture medium is the most common marker for loss of membrane integrity (cytotoxicity).

The luminescent LDH assay has adequate sensitivity to detect as few as 10 dead cells.

Periodic sampling of small aliquots (2-5 µl) of culture supernatant provides a method to detect the occurrence of cell death over time from the same sample of cells.

The remaining live population of cells can be used for multiplexing any orthogonal method to confirm cytotoxicity.

The assay has been used to monitor cytotoxicity of individual 3D spheroids, antibody drug conjugates or antibody-dependent cell-mediated cytotoxicity.

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