Getting more information from organoids: A high-throughput assay for human alanine transaminase (hALT)

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Overview

Alanine transaminase released from hepatocytes in three-dimensional (3D) liver culture models may serve as an indicator of cellular injury or reduced viability and a means to assess compound-induced effects.

High-content imaging can provide a wealth of information from 3D cultures, but analysis of material released or secreted by these cultures provides additional data for assessing cytotoxicity and other effects, while leaving the cells intact and available for further analysis.

Introduction

3D culturing of human cells, often multiple cell types grown in formats where they can form multi-layered structures, is enabling new models for research that are considered more biologically relevant than monolayer (2D) cell cultures. Characterization of organoid responses to candidate drug treatment using imaging and other high-content assay methods is a powerful research tool that provides a wealth of detailed information. However, quantitative methods like the enzyme-linked immunosorbent assay (ELISA) continues to be a route by which information on additional biomarkers serving as indicators of health or disease conditions can be collected non-destructively while preserving the original 3D structures for further studies.

Here we demonstrate the use of a 384-well quantitative assay for human ALT in liver organoid culture supernatant. This single-wash assay could be completed in 90 minutes, enabling a high-throughput workflow applicable to screening. Individual organoids grown in wells of a 384-well microplate were assayed for the release of ALT, which serves as an indicator of decreased liver cell viability in response to drug treatment. Quantitative analysis of results allowed assessment of the effects of a set of relevant compounds on ALT release as a marker of cell viability.

Methods

Process automation

Liver organoids provided by InSphero were grown in Akura™ 384 Spheroid Microplates, with media exchange, compound addition, and supernatant collection performed using a Hamilton Microlab® STAR™ Liquid Handler. A PreciseFlex 400 sample handler (Brooks) and Genera scheduling software (Retisoft) were used to transfer the microplate from incubator to liquid handler and back.

Organoid treatment and sample collection

Compound additions were done from a 96-well master plate to quadruplicate assay wells of the 384-well culture plate. Organoids were incubated in compounds for five days, after which 25 µL of supernatant were collected from each well and transferred to a 384-well polypropylene microplate for short-term storage.

Abcam hALT SimpleStep™ ELISA

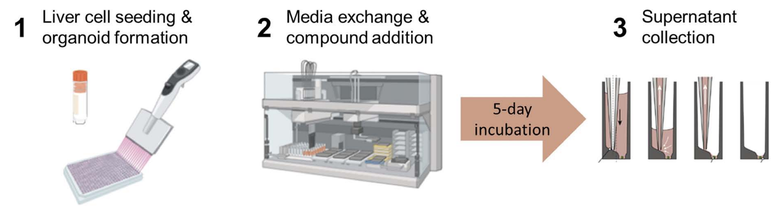
Supernatant from each well was diluted 1:2 in sample diluent and then added to wells of the assay plate. Antibody cocktail was added and the plate incubated for one hour with shaking. Wells were washed using the AquaMax® 4000 Microplate Washer with 384-well wash head, and TMB development solution was added to all wells. Before standard wells reached OD600 of 1.0, stop solution was added, and the absorbance of all wells was read on a SpectraMax® ABS Plus Microplate Reader.

Analysis of hALT released

From the hALT standard curve, the concentration of hALT present in liver organoid supernatants was interpolated using SoftMax® Pro Software. Where sufficient replicates yielded quantifiable hALT levels, results were plotted and compound EC_{50} values were calculated by the software.

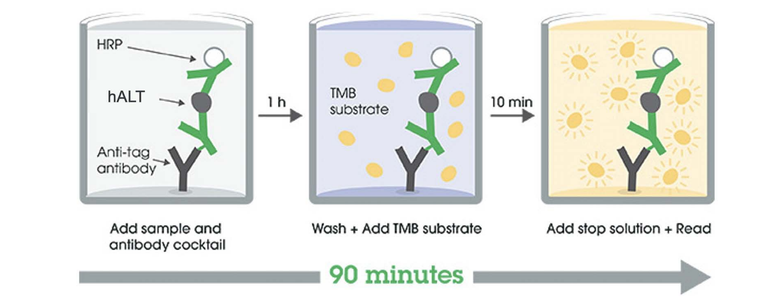
Organoid treatment & assay workflow

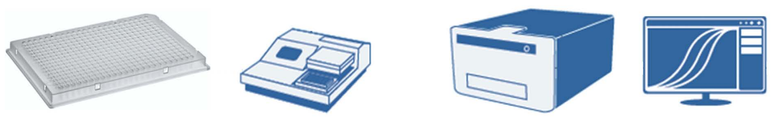
Compound treatment & sample collection



Automation of sample handling. Upon seeding of cells and formation of organoids, automation of media exchange, compound addition, and supernatant collection in 384-well format was performed using an automated liquid handling system.

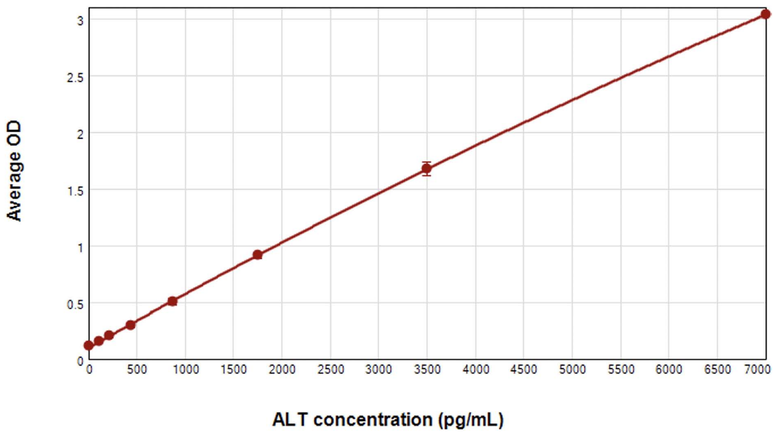
384-well human ALT SimpleStep ELISA





High-throughput ELISA. The SimpleStep ELISA in 384-well format accommodated the 384-well experimental layout used to culture and treat liver organoids. 25-µL supernatants from each well were collected and diluted 1:2 prior to assay with the human ALT ELISA. A single wash step was performed with the AquaMax 4000 Microplate Washer equipped with a 384-well wash head that enabled simultaneous, rapid washing of all wells. Detection was performed with the SpectraMax ABS Plus reader, which reads 8 wells at once for shorter read times.

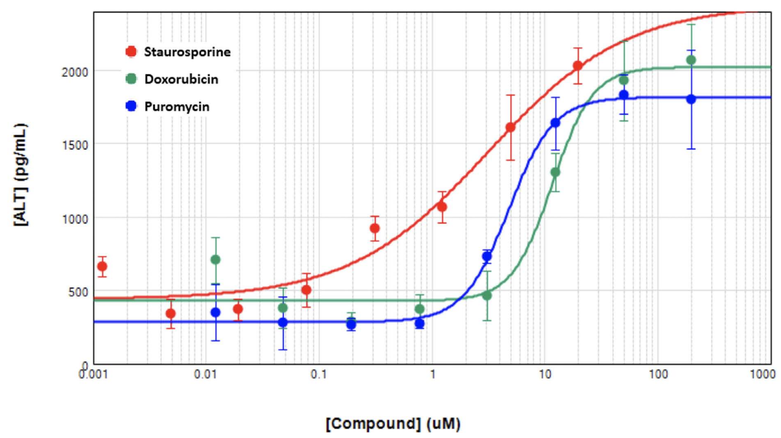
Assay sensitivity & range

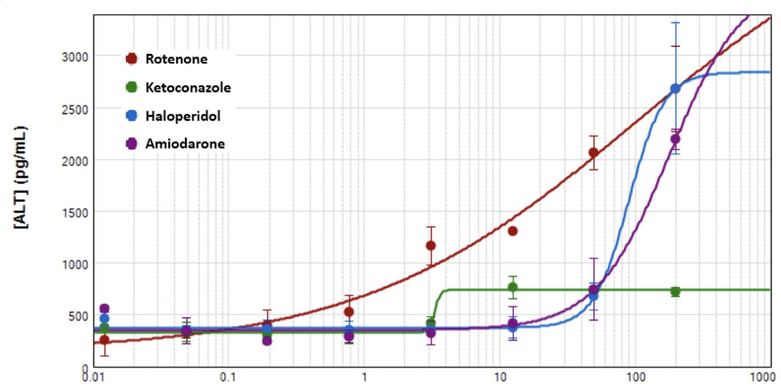


hALT standard curve. The SimpleStep ELISA in 384-well format facilitated the quantitation of 100 to 7000 pg/mL of hALT. Standards were plotted using the quadratic curve fit in SoftMax Pro Software.

Results

Quantitation of hALT released by liver organoids





Compound	EC_{50} (µM)
Staurosporine	3.1
Ketoconazole	3.3
Puromycin	4.8
Doxorubicin	11.6
Haloperidol	89.9
Rotenone	102.1
Amiodarone	179.6

Compound-induced hALT levels & EC_{50}. Human ALT concentrations were determined from the standard curve and plotted vs. compound concentration, with 4-parameter curve fitting performed and EC_{50} values calculated using SoftMax Pro Software.

Conclusion

The 384-well quantitative hALT ELISA from Abcam provides the means to get more information from organoid models grown in a 384-well format. Here we show how culture supernatant can be assayed, leaving the organoids available for further interrogation with high-content imaging or other methods.

The assay's sensitivity enables the use of small volumes of precious sample. Automation of the liquid handling steps involved in organoid culture and supernatant sample collection assures greater reproducibility. Together with the SpectraMax ABS Plus reader and SoftMax Pro software, hands-on and analysis time is decreased for faster results.

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