Day 5: Downstream Cell-Based Assays

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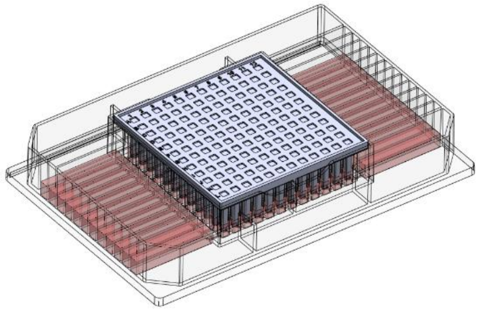
Innovation in Organoid Culture and Disease Modeling

Goal: Provide operation-level training on cell viability assays and downstream workflows used with pillar/perfusion plates.

Technical overview of compatible assays:

- Cell viability (CellTiter-Glo for ATP measurement)
- Fluorescence-based, high-content imaging assays (Calcein AM, TMRM, etc.)
- Immunofluorescence (IF) staining of spheroids/organoids
- RT-PCR

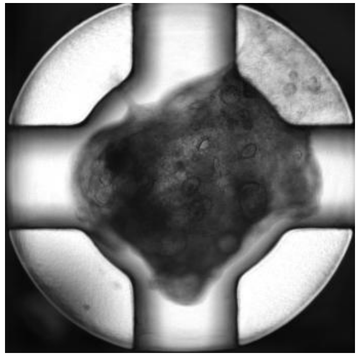
Compatible with Common Lab Equipment for Organoid Analysis

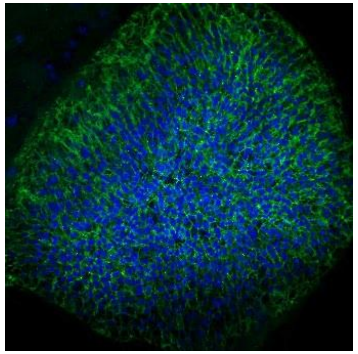


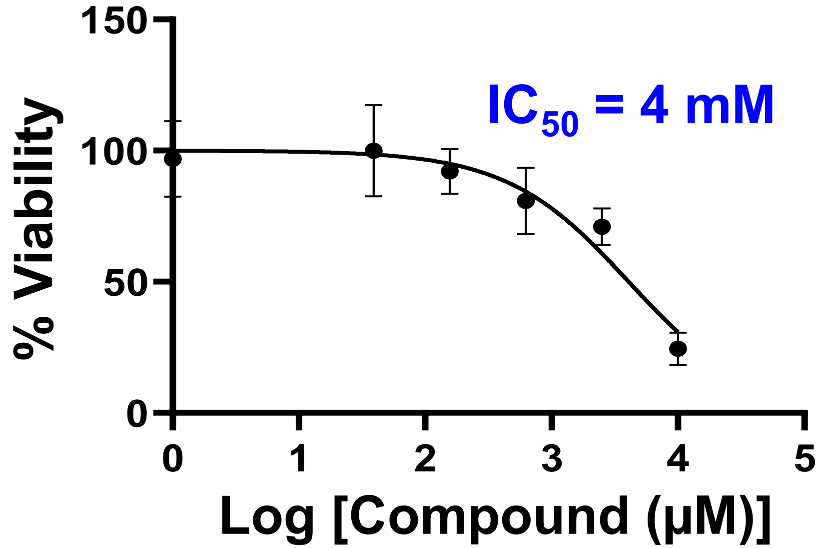
Pillar/perfusion plate

Well plate reader

Fluorescence microscope







The pillar/perfusion plate is fully compatible with standard laboratory equipment for organoid analysis, eliminating the need for customers to invest in additional instrumentation.

Example of Cell Viability Assays on Pillar Plate

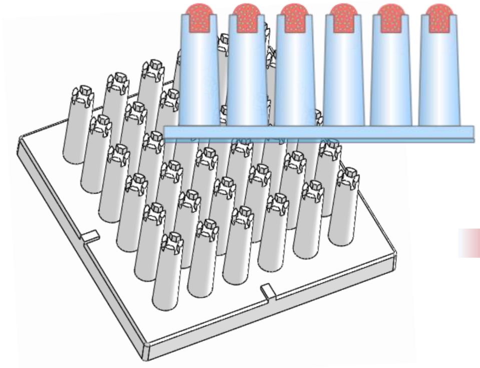
Calcein AM fluorescent dye (ThermoFisher, C1430)

- Measures cell membrane integrity

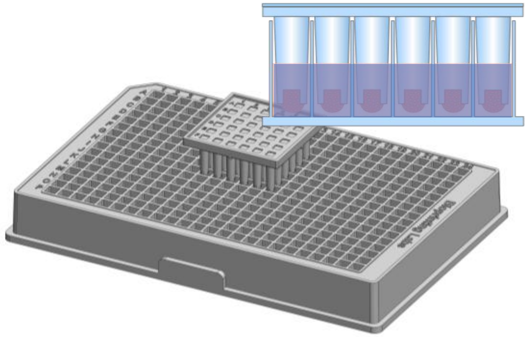
CellTiter-Glo 3D reagent (Promega, G9683)

- Quantifies ATP levels in metabolically active cells

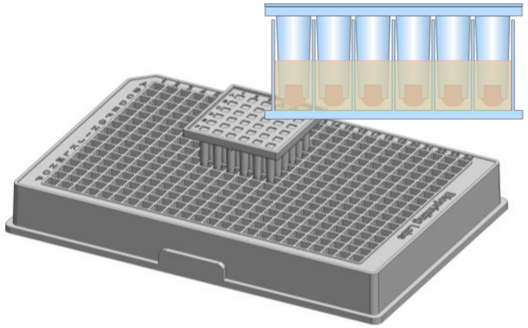
Cell Viability Measurement with Calcein AM



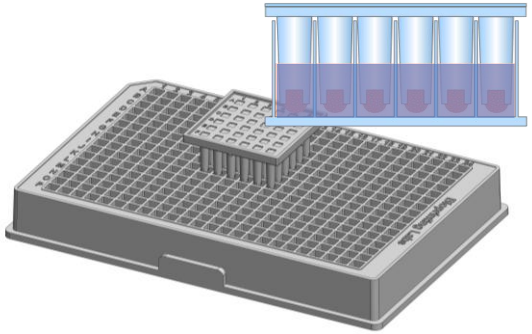
36PillarPlate with cells/organoids



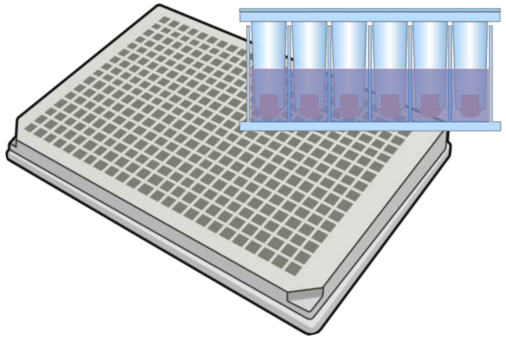
Rinse twice for 15 mins with DPBS in 384DeepWellPlate



Stain cells with calcein AM in 384DeepWellPlate for 90 mins



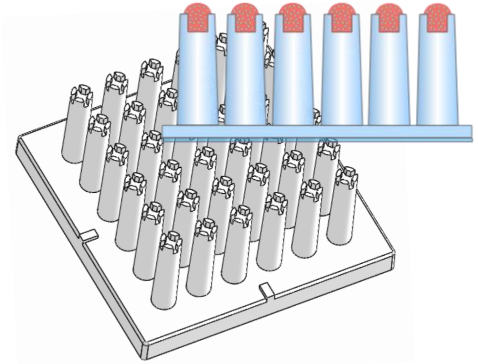
Rinse twice for 20 mins with DPBS in 384DeepWellPlate



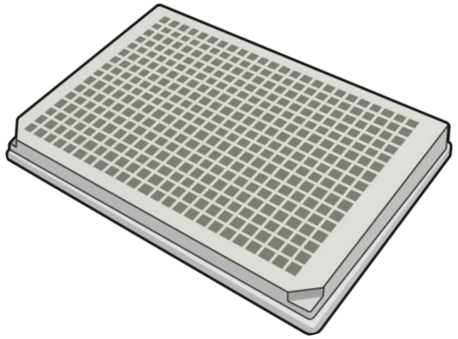
Insert in regular 384-well plate with DPBS for imaging

Image acquisition with automated fluorescence microscope

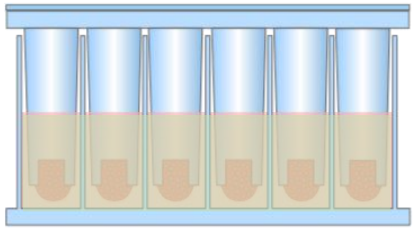
Cell Viability Measurement with CellTiter-Glo



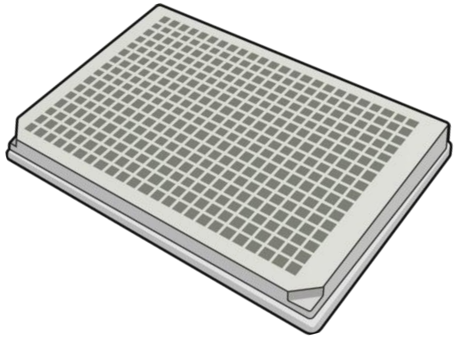
36PillarPlate with cells



Opaque 384-well plate with CellTiter-Glo reagent

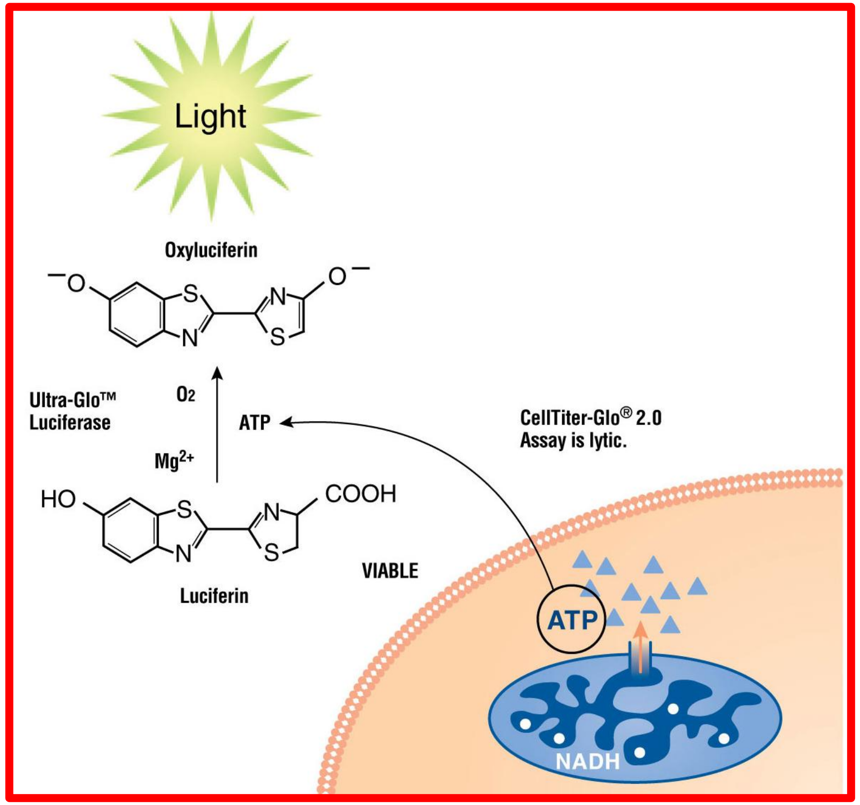


Incubation with CellTiter-Glo for 30 - 45 mins

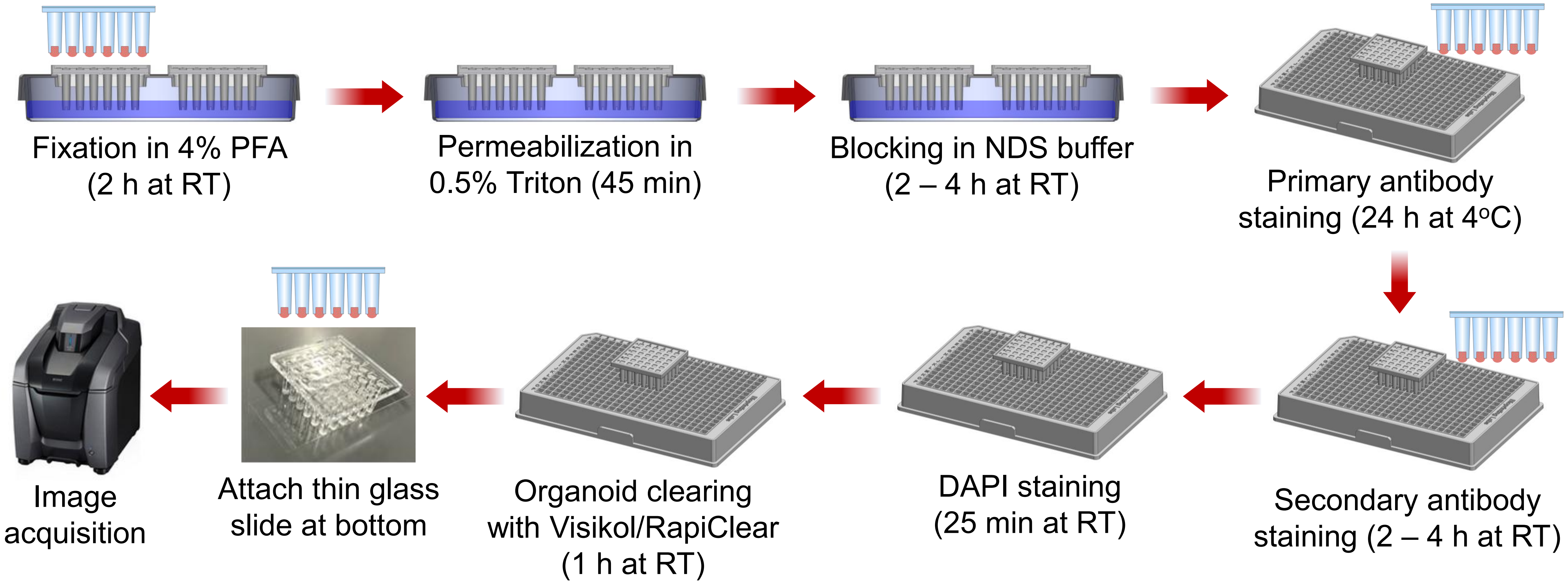


Separation of the opaque 384-well plate

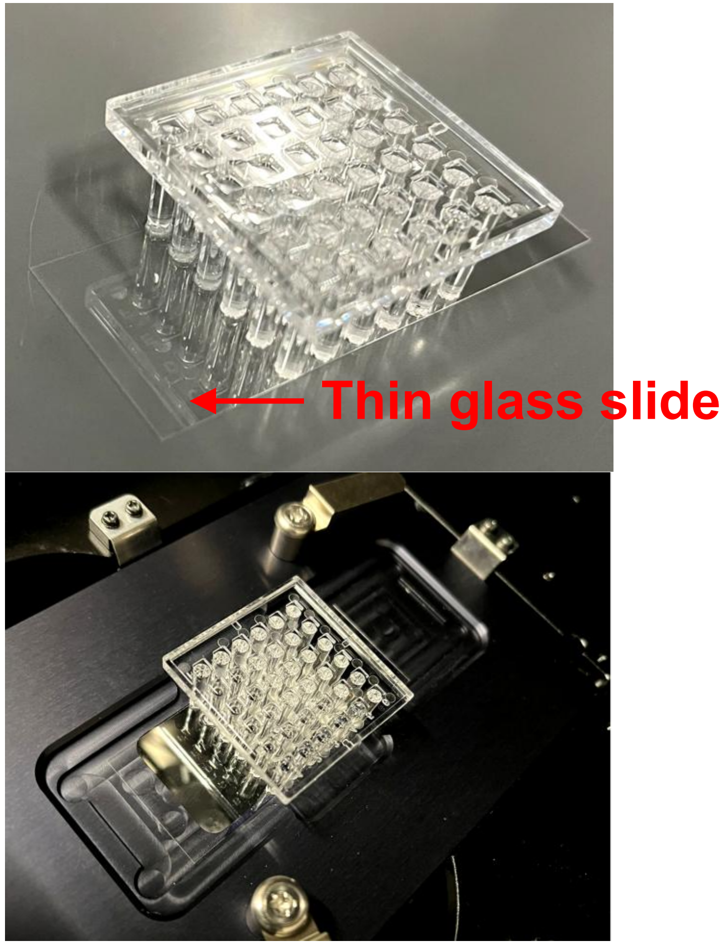
Luminescence measurement at 560 nm



In Situ Immunofluorescence (IF) Staining on Pillar Plate

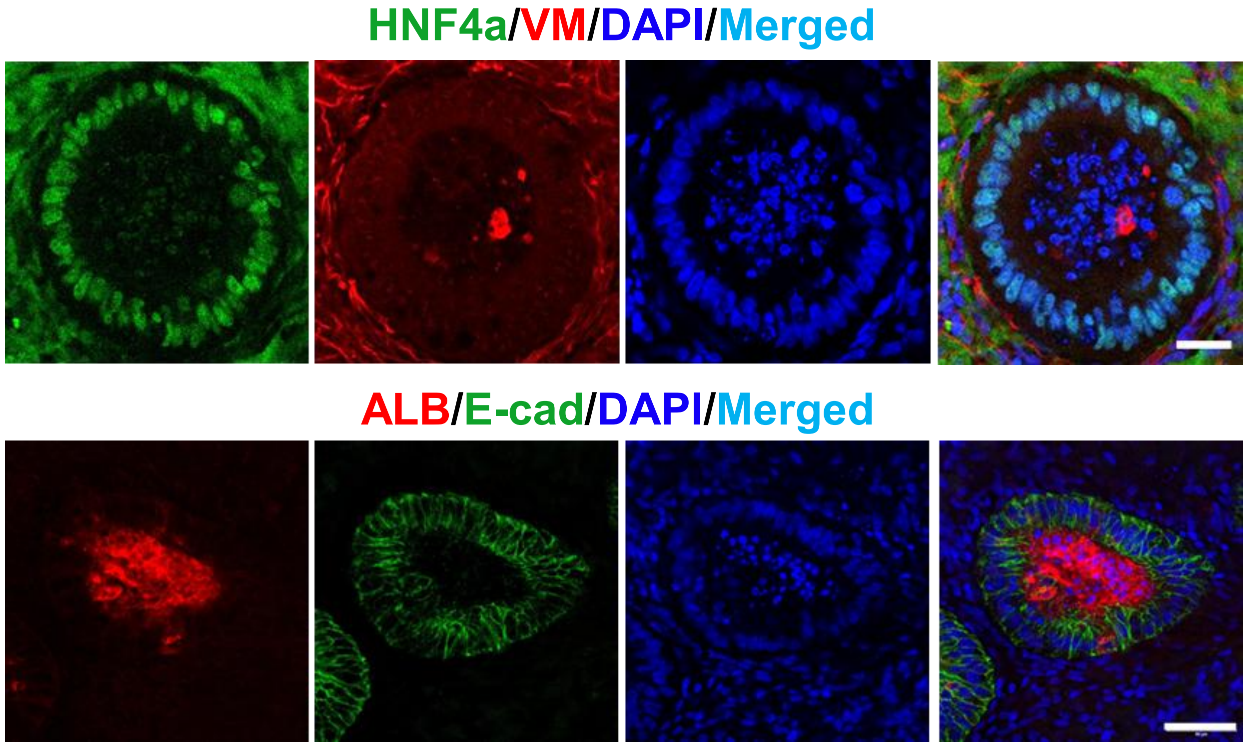


In Situ Whole Mount Organoid Imaging



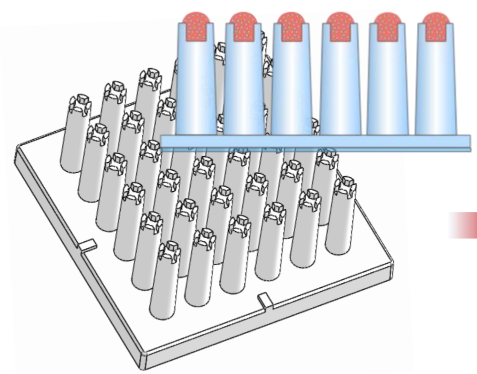
The thin microscopic glass slide attached to the pillar plate with organoids for image acquisition.
DOI: 10.1007/7651_2024_603

Human liver organoids

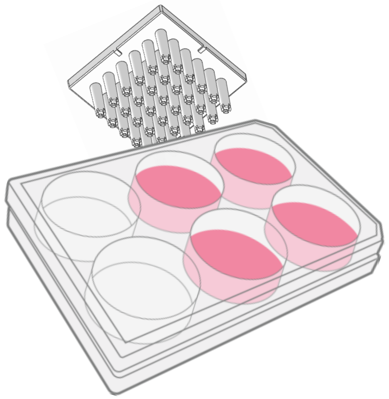


Whole mount immunofluorescence staining of day-25 human liver organoids (HLOs) generated on the pillar plate. Scale bars: 50 µm. DOI: 10.1039/D4LC00149D

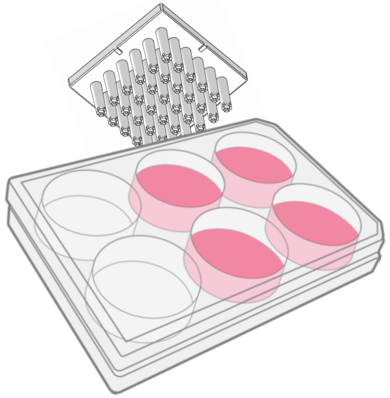
RT-qPCR with Organoids on Pillar Plate



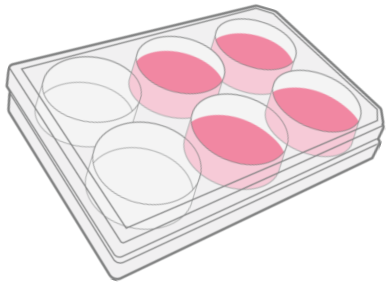
36PillarPlate with cells/organoids



Incubate with cold basal medium for 5 mins in 6-well plate



Incubate with cold organoid harvesting solution for 45 - 60 mins at 4°C in 6-well plate



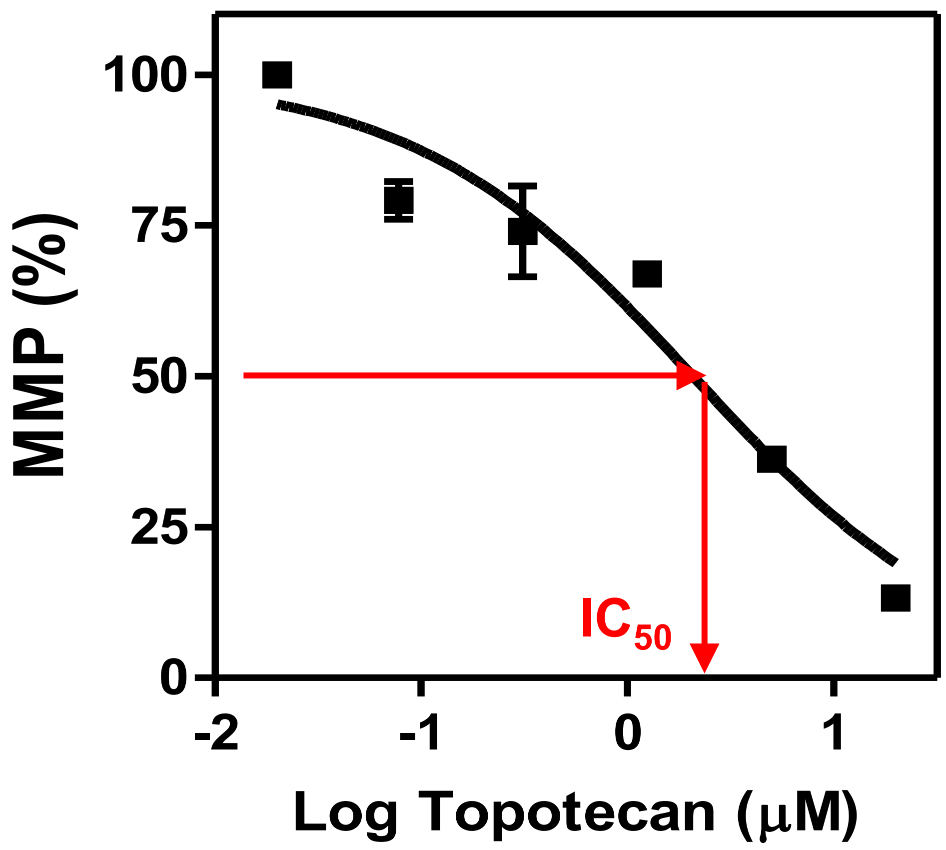
6-well plate with detached organoids ready for RNA extraction

RNA Isolation

cDNA Conversion

qPCR analysis

Sigmoidal Dose-Response Curve and IC_{50} Value



The sigmoidal dose-response curves (variable slope) and IC_{50} values (i.e., concentration of the compound where 50% of cell viability/growth inhibited) were obtained using the following equation:

$$\mathbf{Y = Bottom} + \left[\frac{\boldsymbol{Top - Bottom}}{1 + 10^{(\boldsymbol{Log IC_{50} - X) \times H}}}\right]$$

where IC_{50} is the midpoint of the curve, H is the hill slope, X is the logarithm of test concentration, and Y is the response (% live cells), starting from the top plateau (Top) of the sigmoidal curve to the bottom plateau (Bottom).

The dose-response curve of topotecan from the tetramethyl rhodamine methyl ester (TMRM) assay with ReNcell VM on the 384-pillar plate. The mitochondrial membrane potential (MMP) signals were obtained by calculating the mean and standard deviation (error bars) of six biological replicates, which were normalized with the signals from the DMSO alone control. The IC_{50} value is the compound concentration that interrupts the MMP activity by 50%.

Coefficient of Variation (CV) for Variability Analysis

- For robust assay development on the pillar/perfusion plate platform, it is important to measure the range of errors and plate-to-plate and day-to-day reproducibility.

- The coefficient of variation (CV) is commonly measured to evaluate error ranges of an assay.
- The CV is defined as the ratio of the standard deviation (SD) to the average (Avg) of the signals.

$$\mathbf{CV} = \frac{\boldsymbol{SD}}{\boldsymbol{Avg}} \times 100$$

It is the extent of variability in relation to the average of the signal, thus the inverse of the signal-to-noise ratio. The range of acceptable CV is typically below 25%.

Z’ Factor Analysis for Robustness of the Assay

Instead of using signal to noise (S/N) or signal to background (S/B), the Z’ factor is the most widely used to measure the robustness of a new assay. The Z’ factor is defined as the following equation.

$$Z' = \frac{(Avg_{Max} - 3SD_{Max}) - (Avg_{Min} + 3SD_{Min})}{Avg_{Max} - Avg_{Min}}$$

where Avg_{Max} is an average of maximum fluorescence intensity from fully viable cells on the 384-pillar plate (DMSO control), Avg_{Min} is an average of minimum fluorescence intensity from the cells treated by the highest dose of a compound, SD_{Max} is a standard deviation of maximum fluorescence intensity, and SD_{Min} is a standard deviation of minimum fluorescence intensity.

Assays with a Z’ factor between 0.5 and 1.0 are considered excellent. In addition, assays with a Z’ factor between 0 and 0.5 are considered marginal, and assays with a Z’ factor below 0 are essentially useless. The Z’ factor is used to identify the quality of a new assay prior to testing a large numbers of compounds using high-throughput screening (HTS).