RASTRUM

PhenoVue™ *in situ* Live/Dead Cell Viability Protocol

PHENOVUE™ IN SITU LIVE/DEAD CELL VIABILITY

Highlights

- Viability of HepG2 cells printed in **RASTRUM™** High Throughput models assessed *in situ*.
- Easy-to-perform viability assay enables to determine the health of 3D advanced cell models.
- The combination of **RASTRUM™** Advanced Cell Models and Revvity PhenoVue™ Live/Dead Cell Viability Assay Kit assays provide researchers a high-throughput model to study growth of primary hepatocytes.

Introduction

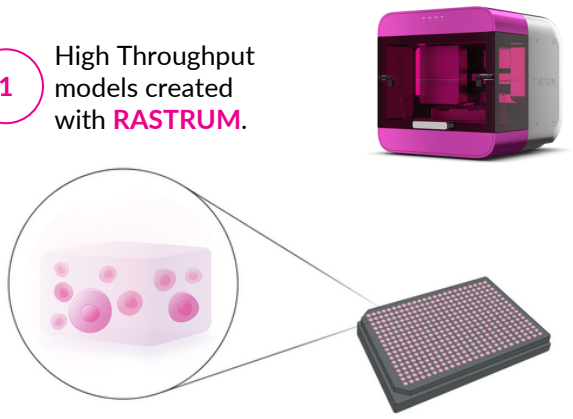
Assessing the viability of cells within **RASTRUM** 3D cell models is a fundamental practice for determining the health of 3D cell cultures. Additionally, viability assessments serve as the foundation for toxicology and efficacy studies of malignant cells.

Through the use of high content imaging, researchers are able to visualize and/or quantify the effects of compounds, growth factors and ECM modifications. Recently, research has trended toward the use of *in situ* analysis of cell cultures to retain spatial information.

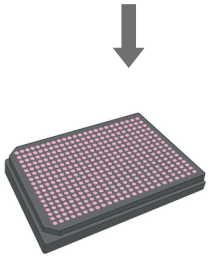
In this protocol, we describe in situ staining of live advanced cell high-throughput models created with **RASTRUM** (HepG2 spheroids) treated with 50mM acetaminophen using PhenoVue™ Live/Dead Cell Viability Assay Kit (Revvity). While this protocol references the HTP model, it can be easily adapted for other **RASTRUM** 3D Advanced Cell Models.

Graphical Protocol

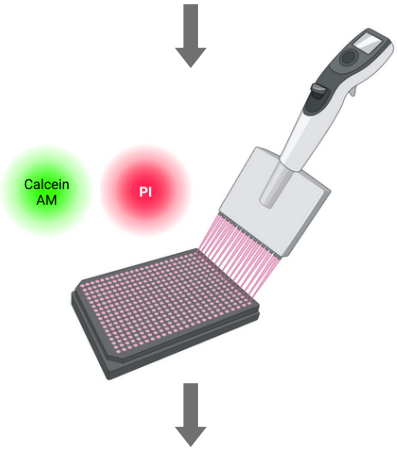
1 High Throughput models created with **RASTRUM**.



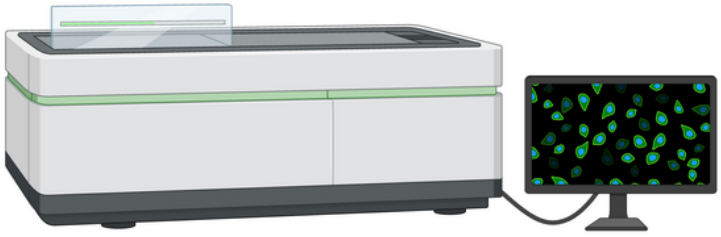
2 Routine cell culture performed.



3 PhenoVue Live/Dead Assay performed.



4 Perform fluorescence imaging on a high content imager (i.e. Opera Phenix).



Protocol

Reagent preparation

1. Equilibrate reagents from PhenoVue™ Live/Dead Cell Viability Assay Kit at room temperature for approximately 30 mins.
2. Reconstitute a vial of Calcein-AM with 20uL DMSO (provided in kit). Incubate at room temperature for 10 minutes.
   Note: Propidium Iodide is provided as a solution and does not require reconstitution.
3. Prepare a working solution of Calcein-AM/Propidium Iodide by adding 1 µL Calcein-AM and 1 µL of Propidium Iodide to 998 µL Assay buffer.
   *Note: Required working solution per well is described in **Table 1**. Adjust final working solution volume and dye concentrations as per experimental needs. Make working solutions fresh and use within 2 hours for best results.*
4. Return stock solutions to -20°C for storage.

PhenoVue™ Live/Dead Cell Viability Assay

1. Aspirate media* from the advanced cell model well plate, leaving media volume as indicated in Table 1.
   Note: Avoid disturbing the hydrogel by aiming the tip to the bottom corners for liquid aspiration.

	96-well	384-well
Well media	*100µL*	*25µL*
Working stock staining solution	*100µL*	*25µL*

Table 1. Media and staining volume requirements (1 µL/well) by well plate format.

2. Add working stock staining solution to each well at volumes indicated in **Table 1**. Allow to incubate for 30 minutes in a 37°C humidified incubator.
   Note: Incubation time may need to be determined in dependent manner, as guided by the manufacturer's recommendations. Confirm the dye working stock is in contact with the hydrogel, whereby pulse centrifugation may be required.

PhenoVue™ Live/Dead Cell Viability Assay

3. After 30 minutes of incubation, remove the plate from the incubator.
4. Proceed to imaging on a fluorescence microscope such as Opera Phenix™ High Content Imaging system using FITC/TRITC filters.
   Note: As staining solution is not removed after the 30 minute incubation time, the intensity of stains may change over time. Image within 60 minutes of stain incubation for best results.

Results

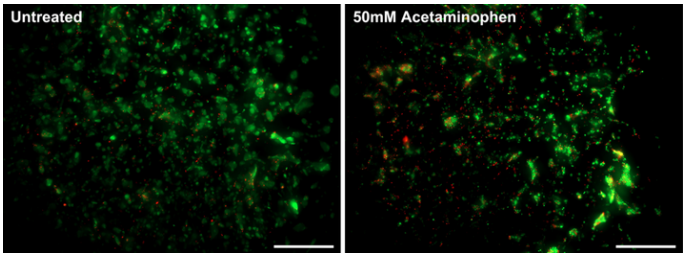


Figure 1. Acetaminophen-treated HepG2 spheroids in RASTRUM matrices showed loss of 3D spheroid structure and more dead cell staining (red) than live cells (green). HepG2 cells (6.25 x 10^6 cells/mL, 1.1 kPa RASTRUM matrix, HTP model) were treated with Acetaminophen on day 1 post-printing for 48 hours. Models were imaged after 30 minutes dye incubation, on a Zeiss Celldiscoverer7 using 5x wide field objective and AF488 and AF555 filter. Images presented as a maximum projection of a z-stack (100µm intervals, 700µm total distance). **Scale bar = 500µm**.

Reagents and Consumables Required

Product name	Catalogue number	Company
RASTRUM™ Matrix	*inquire for details*	*Inventia Life Science*
RASTRUM™ Model	*inquire for details*	*Inventia Life Science*
PhenoPlate™ -96 or -384-well microplates	*6055302, 6057300*	*Revvity*
PhenoVue™ Live/Dead Cell Viability Assay Kit	*PCVA11*	*Revvity*

Equipment Required

Product name	Company
RASTRUM™ Instrument	*Inventia Life Science*
High content imager with FITC/TRITC filters (i.e. Opera Phenix)	*Perkin Elmer, Multiple options*
Multichannel pipette	*Multiple options*
Biosafety cabinet (BSC)	*Multiple options*

References

1. PhenoVue™ Live/Dead Cell Viability Assay Kit Product Information, Perkin Elmer, Document ID: 839911

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