Assays Optimized for 3D Cell Culture

3D Cell Culture Models

3D cell culture model systems mimic tissue-like structures more effectively than monolayer (2D) cell cultures and are used as a more biologically relevant model for studying cell processes. Most existing cell-based assays were designed for 2D cell culture, and must be evaluated and optimized for use in 3D systems.

Optimization and Confirmation of Assay Effectiveness in 3D Models

Promega has performed optimization and confirmation of the effectiveness of many of our robust and sensitive assays on 3D culture models. Assays are optimized to ensure:

- The reagents effectively lyse 3D structures
- The reagents penetrate to the center of 3D spheroids
- The mass of cells does not quench the detection signal before it is detected

Due to the sensitivity of the bright assay signals and robust assay chemistries, a wide array of Promega's cell health and energy metabolism assays can be adapted for use with 3D spheroids by increasing the mixing and/or shaking time after reagent addition.

Caspase-Glo® 3/7 Assay with Optimized Shaking Time

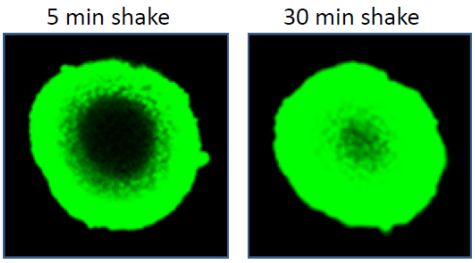

Figure 2. HCT116 spheroids (~330µm diameter) were formed using InSphero GravityPLUS™ hanging drop system. CellTox™ Green Cytotoxicity dye was combined with Caspase-Glo® 3/7 Reagent prior to addition to the spheroids, and samples were shaken for 5 or 30 minutes prior to confocal imaging. The green-stained cells indicates cell lysis.

CellTiter-Glo® 3D Cell Viability and LDH-Glo™ Cytotoxicity Multiplexing in Spheroids

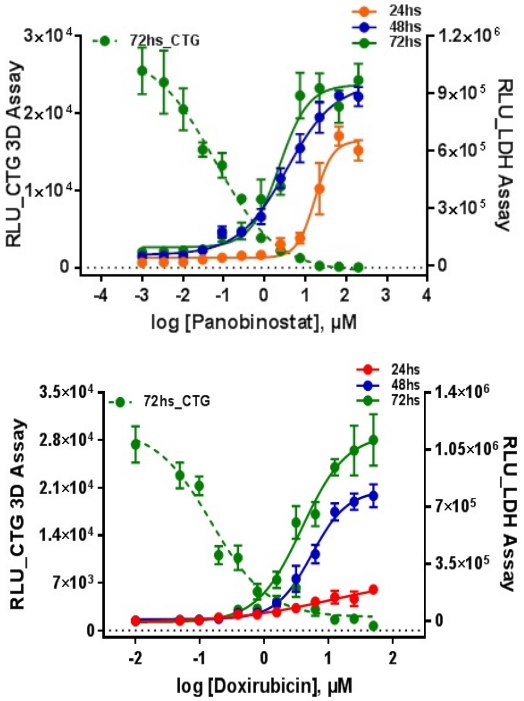

Figure 1. HCT116 spheroids were formed using 2,500 cells/well in Corning® 384 Well Ultra-Low Attachment plates (400µm diameter). Spheroids were treated with increasing concentrations of Panobinostat (top) or Doxorubicin (bottom). At 24, 48 and 72 hours following treatment, 2µl media was removed, diluted 20x in storage buffer, and frozen. Following completion of the time course experiments all diluted media samples were assayed using a 1:1 ratio of diluted sample to LDH-Glo™ Cytotoxicity Assay Reagent. CellTiter-Glo® 3D Cell Viability Assay Reagent was added to the spheroids to determine the amount of live cells remaining.

3D Assay Considerations

When performing assay optimization with 3D models, consider that the small number of cells used in many 3D systems requires sensitive assays. The increased time needed for cell lysis requires detection reagents with signals stable for the extended incubation time. Reagents such as MTT and resazurin may not have the sensitivity, lytic power, or signal robustness for use in a 3D model system.

Due to the wide range of 3D model systems, optimal conditions should be empirically determined for each model system and reagent combination.

Assays Confirmed to be Effective with 3D Model Systems

Promega has confirmed the following assays perform robustly in 3D model systems with protocol adaptation.
Contact **ch_techserv@promega.com** for additional details.

Viability:

CellTiter-Glo® 3D Cell Viability Assay

The reagent is designed for determining cell viability in 3D microtissue spheroids. With enhanced lytic capacity, the assay reagent penetrates large spheroids and allows for more accurate determination of viability.

RealTime-Glo™ MT Cell Viability Assay

A non-lytic method to determine cell viability in real time up to 72 hours by measuring the reducing potential of viable cells.

Cytotoxicity:

LDH-Glo™ Cytotoxicity Assay

Measure LDH released from 3D cell culture systems with a bioluminescent readout. The assay is non-lytic, allowing the spheroids or other cell models to be used for additional applications.

CellTox™ Green Cytotoxicity Assay

The assay measures changes in membrane integrity that occurs as a result of cell death using a dye that preferentially stains the dead cells' DNA, generating a fluorescent signal. The assay can be used for real time kinetic measures of cell death and multiplexed with bioluminescent assays to give a complete picture of spheroid health.

Autophagy:

Autophagy LC3 HiBiT Reporter Assay

Quantitatively measure autophagic flux with a luminescent LC3 reporter. HEK293 reporter cells can be used to prepare 3D spheroids or the reporter can be expressed in a different model.

Apoptosis:

Caspase-Glo® 3/7 Assay

Detect caspase 3/7 activity in spheroids using the reproducible, luminescent assay by increasing the shaking time after reagent addition to improve cell lysis.

RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay

A no-wash, one-step Annexin V binding assay that is non-lytic allows for continual monitoring of the cell state to determine apoptotic onset in spheroid models.

Energy Metabolism:

Metabolite Detection Assays

Detect lactate, glucose, glutamine and glutamate in cell culture media from 3D microtissue culture wells. Monitor events like changes to glycolysis or glutaminolysis by the spheroids.

GSH/GSSG-Glo™ Assay

Assess toxicological or oxidative stress by determining the ratio of reduced to oxidized glutathione.

P450-Glo® Assay Systems

Measure the metabolic activity of spheroids using a bioluminescent assay by modifying the protocol with an extended incubation time.

Reporter Assays:

Nano-Glo® Luciferase Assay System

The bright NanoLuc® reporter allows more sensitive detection in challenging applications with add-and-read-simplicity. No need for separate lysis and reagent injection steps.

ONE-Glo® Luciferase Assay System

The highly sensitive, robust assay detects firefly luciferase reporter gene expression in 3D models.

For more information about use of Promega's assays with 3D models, visit:
www.promega.com/3DPoster

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