

Assays Optimized for 3D Cell Culture

3D cell culture model systems mimic tissue-like structures more effectively than monolayer (2D) cell cultures and are used as more biologically relevant models 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

Many of our assays have been optimized to ensure:

- Reagents effectively lyse 3D structures
- Reagents penetrate to the center of 3D spheroids
- Large mass of cells does not quench the signal before detection

Due to the sensitivity of the bright assay signals and robust assay chemistries, a wide array of our 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 3D Assay: Verified with Matrigel® Embedded Cultures

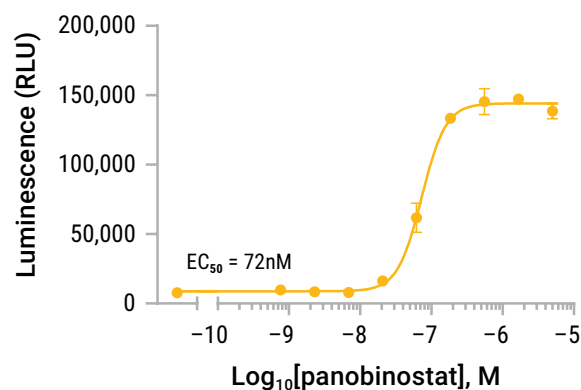


Figure 1. HCT116 cells were embedded in a 4.5mg/ml Matrigel® Solution and allowed to grow for 4 days, then treated with a serial dilution of Panobinostat. Caspase-Glo® 3/7 3D Assay Reagent was added to determine caspase induction.

Explore webinars, articles and other 3D culture resources at www.promega.com/3DGuide

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

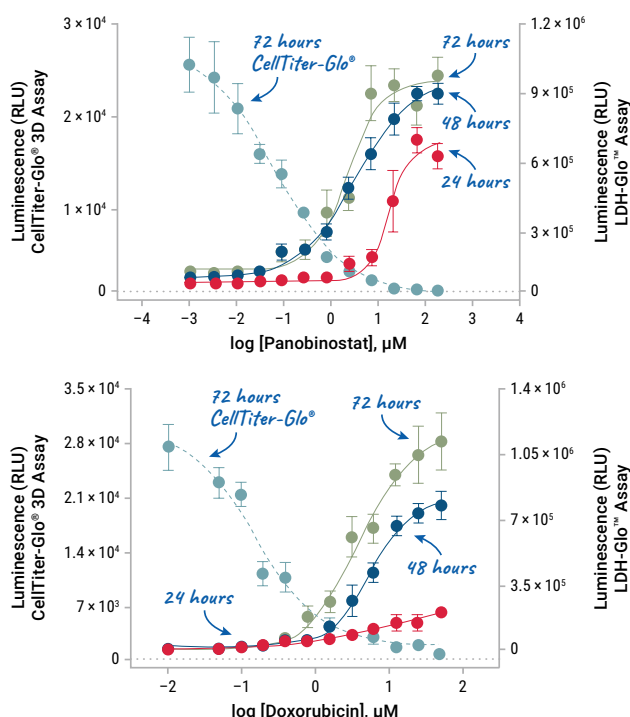


Figure 2. HCT116 spheroids were formed using 2,500 cells/well in Corning® 384 Well Ultra-Low Attachment plates (400μm diameter). Spheroids were treated with increasing concentrations of Panobinostat (top) or Doxorubicin (bottom). At 24, 48 and 72 hours following treatment, 2μl media was removed, diluted 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 number of live cells remaining.

3D Assay Considerations

When optimizing assays with 3D models, consider:

- The small number of cells used in many 3D systems requires sensitive assays.
- The extended incubation time needed for cell lysis requires detection reagents with high signal stability.
- Traditional reagents such as MTT and resazurin may not have the sensitivity, lytic power, or signal robustness for use in 3D models.
- Due to the wide range of 3D model systems, optimal conditions should be empirically determined for each model system and reagent combination.

Confirmed Assays Effective in 3D Model Systems

We have confirmed the following assays perform robustly in 3D model systems with protocol adaptation:

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.

(Cat. # G9681, G9682, G9683)

RealTime-Glo™ MT Cell Viability Assay

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

(Cat. # G9711, G9712, G9713)

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. (Cat. # J2380, J2381)

CellTox™ Green Cytotoxicity Assay

The assay measures changes in membrane integrity caused by cell death using a dye that preferentially stains dead cell 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. (Cat. # G8741, G8742, G8743)

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. (Cat. # GA2550, GA1040, GA1050)

Apoptosis

Caspase-Glo® 3/7 3D Assay

Detect caspase 3/7 activity in spheroids using reagents validated for use with multiple 3D models, including spheroids and cells embedded in Matrigel®.

(Cat. # G8981, G8982, G8983)

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.

(Cat. # JA1011, JA1012)

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 in individual spheroids.

(Cat. # J5021, J6021, J7021, J8021)

Lipid Metabolism Assays

Detect endpoints of lipid metabolism, including glycerol, triglyceride, cholesterol and cholesterol esters.

(Cat. # J3150, J3160, J3190)

P450-Glo® Assay Systems

Measure the metabolic activity of spheroids using a bioluminescent assay by modifying the protocol with an extended incubation time. (Cat. # V8321, V8322, V9781)

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.

(Cat. # N1110, N1120, N1130)

ONE-Glo® Luciferase Assay System

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

(Cat. # E6110, E6120, E6130)

Learn more about our 3D assays at www.promega.com/3DCulture

Need help? Email us: de_techserv@promega.com

