Assaying Cell Health in 3D Cell Culture with the GloMax® Discover System

Promega Corporation

Materials Required

- RealTime-Glo™ MT Cell Viability Assay (Cat.# G9711)
- CellTiter-Glo® 3D Cell Viability Assay (Cat.# G9681)
- GloMax® Discover System (Cat.# GM3000)
- White, 96-well assay plates
- (Corning Cat.# 3903)
- GravityPLUS™ 3D culture and Assay Platform (InSphero CS-06-001)

Caution: We recommend the use of gloves, lab coats and eye protection when working with these or any chemical reagents.

Protocols: GloMax Discover System Technical Manual #TM397, RealTime-Glo™ MT Cell Viability Technical Manual TM#431 and CellTiter-Glo® 3D Cell Viability Technical Manual TM#412 are available at: **www.promega.com/protocols/**

It is widely accepted that cell cultures grown in 3D in vitro arrays more closely mimic physiologically relevant cellular conditions when evaluating cell health for testing and identification of new therapeutics during drug discovery. Traditionally cultured cells have been grown in culture flasks using treated surfaces to promote cell attachment and optimal conditions for cell growth. However, cells grown under 2D conditions do not always mimic cells from living tissues. Several commercial technologies exist to culture cells in 3D for researchers to study cellular responses during drug development and establish more predictive model systems. Such conditions provide the intricate matrix interactions, communication, and growth conditions to that of tissue cells in vivo.

The InSphero GravityPLUS™ 3D Culture and Assay Platform is a scaffold-free 3D culture system designed to grow microtissues from hanging drops. The system contains plates to culture and assay microtissues. The Promega CellTiter-Glo® 3D and RealTime-Glo™ MT Cell Viability assays effectively assay for metabolically active cells through the luminescent measurement of cellular ATP and membrane potential, respectively.

Measuring the cell viability from 3D cultured cells can be easily performed using the GloMax® Discover detection system with integrated protocols for CellTiter-Glo® 3D and RealTime-Glo™ to provide an extended dynamic range and superior detection sensitivity. This Application Note describes the protocol to measure cell viability from cultured cells grown the GravityPLUS™ 3D Culture and Assay Platform and the GloMax® Discover System.

Microtissue Formation in the GravityPLUS™ Plate and Trap

For detailed instructions and assay notes for various assay volumes and plate formats, see the GravityPLUS™ 3D Culture and Assay Platform Technical Manual (InSphero Cat.# CS-06-001). The following protocol is performed in 96-well plates.

1. HCT116 cells were harvested, counted and diluted to 3.2×10^5 cells/ml in filtered McCoy's medium with 10% FBS.
2. The cells were two-fold serial diluted in McCoy's medium from 3.2×10^5 cells/ml to 2.5×10^3 cells/ml.

3. A GravityPLUS™ Handing-Drop Plate was prepared as described in the GravityPLUS™ kit manual. A 40ul aliquot of each cell dilution was added to the GravityPLUS™ Hanging-Drop Plate for final cells/well of 100, 200, 400, 800, 1600, 3200, 6400, and 12800.
4. The GravityPLUS™ Hanging-Drop Plates were then placed in a humidity controlled, 37ºC incubator with 5% CO_2 for 4 days.
5. After the 4 days incubation, the cells were transferred to the GravityTRAP™ Plates as described in the GravityPLUS™ kit manual.
6. Microtissue sizes were determined using CellSense software and an Olympus CKX41 microscope with a 4X objective (Figure 1).
7. Media with microtissues were transferred to a 96-well, white, clear-bottom plate (Corning #3903).

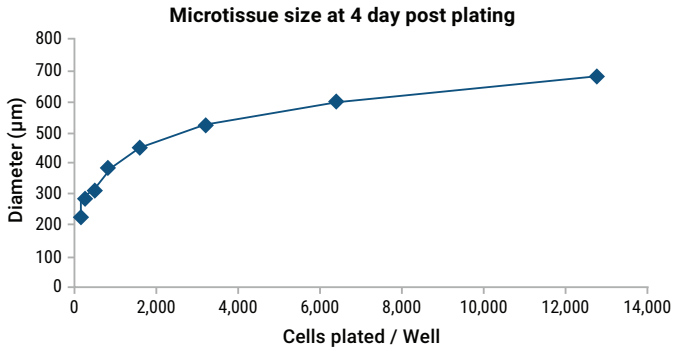

Figure 1. Microtissue diameter correlates with increasing concentrations of plated cells. Microtissues at each plated cell concentration were measured by CellSense software and an Olympus CKX41 microscope containing a 4X objective. Size measurements were plotted for each plated cell concentration.

Cell Viability Assays

For detailed instructions and assay notes for various assay volumes and plate formats, see the *RealTime-Glo™ MT Cell Viability Assay Technical Manual* TM#431 and *CellTiter-Glo® 3D Cell Viability Assay Technical Manual* TM#412. The following protocols were performed in 96-well plates.

1. 2X RealTime-Glo™ Reagent (substrate plus enzyme mixture) was prepared as described in TM #431.
2. A 100µl aliquot of 2X RealTime-Glo™ Reagent was added to all assay wells with the GravityTRAP™ Plate.
3. The cells were then incubated for 60 minutes at 37°C incubator with 5% CO_2.
4. Luminescence was measured using the GloMax® Discover Instrument using the RealTime-Glo™ preset protocol.

Following cell viability assessment using the RealTime-Glo™ MT Cell Viability assay, luminescent signal was plotted for each plated cell concentration (Figure 2).

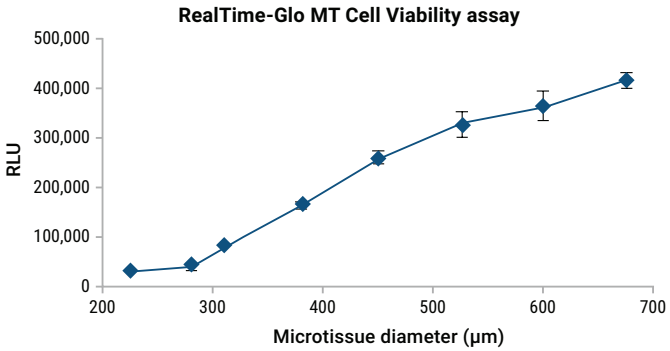

Figure 2. Cell viability from increasing microtissue sizes correlates with luminescent output. Cells were diluted two-fold in McCoy's medium from 3.2×10^5 cells/ml to 2.5×10^3 cells/ml and grown according to the GravityPLUS™ 3D Culture and Assay Platform Technical Manual. RealTime-Glo™ Reagent (100µl) was added to the microtissues. Luminescence was recorded 10 minutes after reagent addition using the GloMax® Discover System. Values present represent the mean of 3 replicates of each cell concentration. Error bars are ±1 standard deviation.

CellTiter-Glo® 3D Cell Viability Assay

1. CellTiter-Glo® 3D Reagent (substrate plus enzyme mixture) was prepared as described in TM#412.
2. CellTiter-Glo® 3D Reagent (100µl) was added to all assay wells with the GravityTRAP™ Plate.
3. The plate was mixed on an orbital shaker for 10 minutes at 600 rpms.
4. The cells were then incubated for an additional 20 minutes at room temperature.
5. Luminescence was measured using the GloMax® Discover instrument using the CellTiter-Glo® preset protocol.

Following cell viability assessment using the CellTiter-Glo® 3D Cell Viability assay, luminescent signal was plotted for each plated cell concentration (Figure 3).

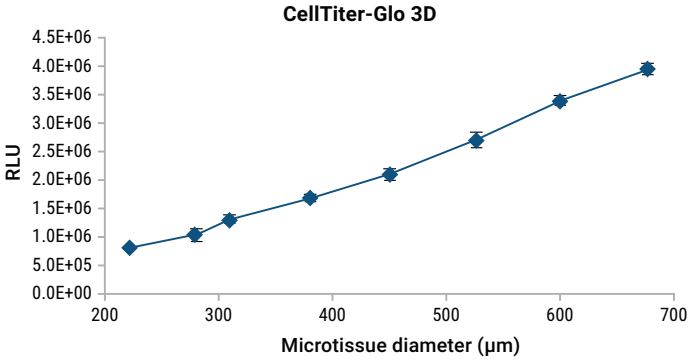

Figure 3. Cell viability from increasing microtissue sizes correlates with luminescent output. Cells were diluted two-fold in McCoy's medium from 3.2×10^5 cells/ml to 2.5×10^3 cells/ml and grown according to the GravityPLUS™ 3D Culture and Assay Platform Technical Manual. CellTiter-Glo® 3D Reagent (100µl) was added to the microtissues. Luminescence was recorded 10 minutes after reagent addition using the GloMax® Discover System. Values present represent the mean of 3 replicates of each cell concentration. Error bars are ±1 standard deviation.

Conclusion

Microtissue cell viability showed a direct correlation between microtissue size and luminescent output using two cell viability assays with the GloMax® Discover System. The RealTime-Glo™ and CellTiter-Glo® 3D cell viability assays together with GloMax® Discover provide strong assay sensitivity capable of detecting various size microtissues prepared using the GravityPLUS™ 3D Culture and Assay Platform. The GloMax®Discover was developed, optimized and integrated with Promega cell viability assays to provide a sensitive, flexible and easy-to-use system to study cell health in vitro.