MO:BOT-Driven Automated Workflow for 3D Cell Culture Model Generation and Viability Assessment Using CellTiter-Glo® 3D Cell Viability Assay

Application Note

May 2026

MO:RE GmbH
Martinistraße 64
20251 Hamburg, Germany
+49 40 23834412
dana@more.science
www.more.science

Promega Corporation
2800 Woods Hollow Road
Madison, WI 53711, USA
+1 800 356 9526
techserv@promega.com
www.promega.com

Abstract

Three-dimensional (3D) cell culture models, including spheroids and organoids, offer superior physiological relevance compared to 2D systems but remain challenging to scale and standardize [1, 2]. Beyond the complexity of the models themselves, manual handling steps between culture and downstream assays introduce significant variability, limiting reproducibility and complicating protocol transfer across laboratories and platforms.

To address these limitations, we integrated the CellTiter-Glo® 3D Cell Viability Assay into a fully automated, end-to-end workflow on the MO:BOT platform, enabling both 3D model generation and ATP-based viability assessment within a single system [3]. The homogeneous, luminescent CellTiter-Glo® 3D assay (Promega) is inherently compatible with automation and supports robust viability measurements across diverse 3D models, including iPSC-derived cortical, midbrain, and kidney organoids, as well as hepatic spheroids derived from cell lines [4, 5].

By eliminating manual intervention between culture and assay, the MO:BOT reduces operator-dependent variability and ensures consistent handling conditions across experiments. Automated workflows on MO:BOT consistently yielded higher and less variable CellTiter-Glo® 3D signals compared to manual methods, reflecting greater cell viability resulting from standardized cell seeding, medium exchange, and reagent handling throughout the workflow. This integrated approach enables reproducible, transferable, and quality-controlled 3D culture workflows, positioning the MO:BOT as a scalable solution for preclinical research and screening applications.

Introduction

Organoids and spheroids better capture tissue architecture, cell–cell interactions, and organ-specific functions than 2D systems, supporting more predictive toxicity testing and disease modeling [1, 2, 6]. However, manual media exchanges, dosing, and endpoint assays are labor-intensive, operator-dependent, and difficult to scale, limiting the routine use of complex 3D models [2, 6].

Automation standardizes liquid handling, timing, and environmental control, reducing variability and enabling larger studies [6]. The MO:BOT enables accurate and precise 3D cell seeding, ensuring homogenous organoid and spheroid formation, as well as consistent reagent dispensing throughout the workflow. This is particularly advantageous for ATP-based

viability assays such as CellTiter-Glo® 3D, where uniform cell aggregates and reproducible reagent dispensing, directly affect signal quality [4, 5].

The CellTiter-Glo® 3D Cell Viability Assay quantifies ATP as a surrogate for metabolically active cells and is optimized for multicellular structures. Its single-addition, homogeneous format, enhanced lytic capacity, and stable luminescent signal make it highly compatible with automated workflows [6]. Here, we describe a new MO:BOT workflow that integrates CellTiter-Glo® 3D as a quality control for a broad range of organoids and spheroids, highlighting both the versatility of CellTiter-Glo® 3D assay and the importance of automation in generating 3D cell culture models with consistent viability.

Materials and Methods

The MO:BOT Platform and 3D Models

The MO:BOT, is an automated laboratory platform (mo:re GmbH, Hamburg, Germany) for 3D human cell culture workflows. It combines plug-and-play on-deck modules (heater, cooler, imaging module, shaker) to automate different types of 3D cell culture workflows and use cases. Together with the integrated brightfield imaging system, which acquires and analyzes well-by-well morphological parameters such as area and roundness, the MO:BOT enables a precise execution of cell-based workflows and continuous control and monitoring of culture quality [3].

For this project, the MO:BOT workflow executed:

- Automated cell seeding of the four human 3D *in vitro* models
- Scheduled medium exchanges
- Brightfield imaging for morphological QC
- Automated CellTiter-Glo® 3D Cell Viability Assay reagent addition

The following 3D models were evaluated:

- Hepatic spheroids (HepG2) cultured in 96well ultra-low attachment plates
- iPSC-derived cortical, midbrain, and kidney organoids cultured in 96well ultra-low attachment plates

Automated Workflow

1. **Cell seeding:**
   MO:BOT automatically dispensed an accurate cell density into 96-well ultra-low attachment for the different *in vitro* models. Seeding densities were optimized per model.

2. **Culture maintenance:**
   Full medium exchanges were performed automatically on the MO:BOT every 48-72 h. The cultures were maintained at 37°C, 5% CO_2, and high humidity throughout the experiment. During the culture period, brightfield images were acquired with the integrated imaging module (MO:CROSCOPE) to monitor organoid and spheroid growth.

3. **CellTiter-Glo® 3D Cell Viability Assay (Promega):**
   The assay was applied as an endpoint viability assay, with measurement timepoints scheduled according to the specific maturation and culture duration requirements of each 3D model. The plates were equilibrated to room temperature. The MO:BOT mixed the CellTiter-Glo® 3D Reagent and dispensed a 1:1 ratio to culture medium, plates were shaken for 5 min, and luminescence was recorded after 25 min additional incubation time using a plate reader (1 s integration time per well).

Manual Reference Workflow and Data Analysis

Parallel cultures were handled manually using identical seeding densities, media schedules, and CellTiter-Glo® 3D batch and protocol across all cell culture models. This enabled one-to-one comparison of 3D aggregates' uniformity and viability readouts. Viability data were expressed as:

- Area-normalized RLU (luminescence normalized to spheroid/organoid area)
- Fold change (automated/manual)

Statistical comparisons used unpaired t-tests ($p < 0.05$).

Results

End-to-End Automated 3D Culture Workflow on MO:BOT

The MO:BOT platform automates all critical steps from initial cell seeding through 3D culture maintenance to CellTiter-Glo® 3D assay execution within a single, continuous workflow. By integrating model generation and downstream assay preparation, it eliminates manual handling and intermediate transfer steps that commonly introduce variability in conventional 3D workflows.

This fully automated and controlled process ensures precise execution of key parameters, including timing, media exchange, reagent addition, and incubation conditions. As a result, data consistency and reproducibility are improved not only though precise liquid handling but also through the standardization of upstream culture conditions and downstream assay execution.

The integrated workflow enables reliable viability assessment across diverse 3D models and supports reproducible results that are readily transferable between experiments, users, and laboratory environments.

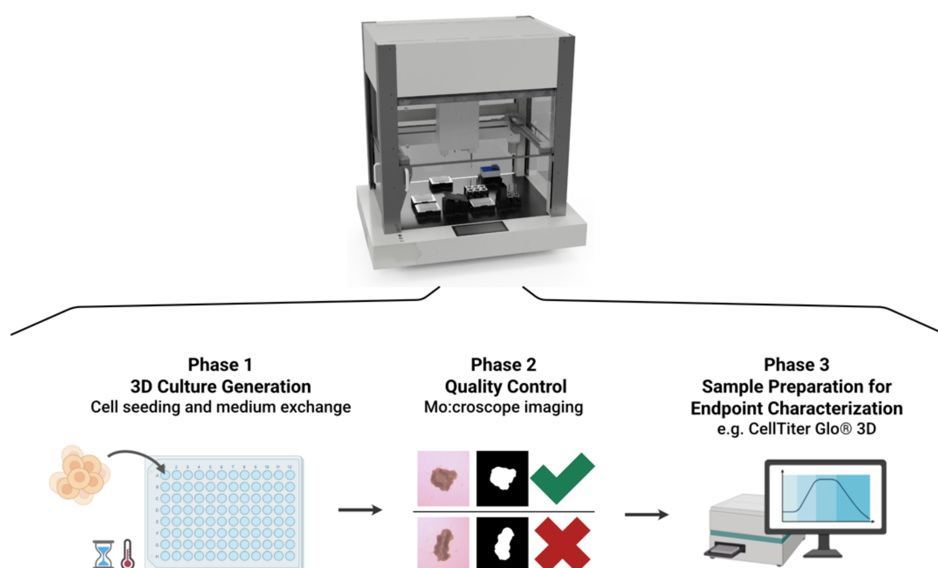


Figure 1: Automated workflow for 3D cell model generation and characterization. The MO:BOT automates cell seeding, medium exchange, brightfield quality control imaging, and endpoint CellTiter-Glo® 3D Cell Viability Assay. Integrated robotic liquid handling ensures reproducible execution of all workflow steps.

Enhanced Viability and Reduced Variability Across 3D Models

The cell viability across four *in vitro* models was assessed and compared to manual handling, with a focus on whether automated generation and handling of spheroids/organoids improves model uniformity and standardization, thereby supporting more robust endpoint readouts.

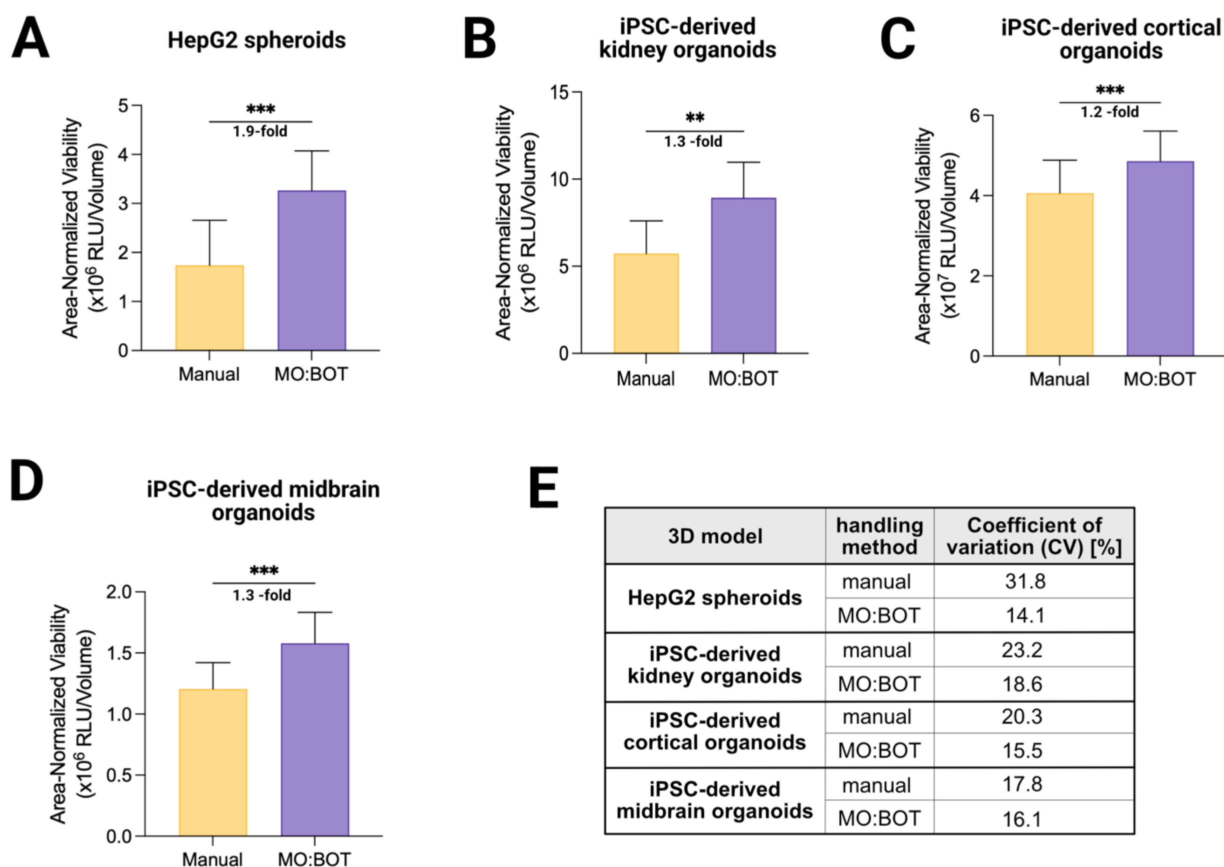
MO:BOT–based HepG2 spheroid culture resulted in markedly higher area-normalized viability compared with manually handled spheroids, with automated conditions yielding approximately 1.3–1.9-fold higher ATP content per spheroid (Figure 2A). Comparable trends were observed for iPSC-derived kidney, cortical, and midbrain organoids, where automated workflows consistently produced higher CellTiter-Glo® 3D signals than the corresponding manually generated structures (Figure 2B–D). Across all four models, the assay delivered robust, interpretable readouts, demonstrating that CellTiter-Glo® 3D can be applied uniformly to both cell line–derived spheroids and sensitive iPSC-derived organoids.

Furthermore, analysis of the area coefficient of variation (CV) in viability measurement (Figure 2E) revealed that the MO:BOT not only increases mean viability but also reduces variability, reflecting the cumulative benefit of automated cell seeding and medium exchange on 3D model homogeneity.

This enhancement reflects that the MO:BOT:

- Executes more consistent medium exchange timing and volumes
- Reduces mechanical stress through gentle, reproducible automated pipetting
- Minimizes exposure to suboptimal environmental conditions during handling
- Eliminates operator-to-operator variability

Together, these results confirm that the combination of MO:BOT automation and CellTiter Glo® 3D Cell Viability Assay provides a broadly applicable, uniform viability readout across diverse 3D human *in vitro* models, while minimizing variability introduced by manual handling.



3D model	handling method	Coefficient of variation (CV) [%]
HepG2 spheroids	manual	31.8
	MO:BOT	14.1
iPSC-derived kidney organoids	manual	23.2
	MO:BOT	18.6
iPSC-derived cortical organoids	manual	20.3
	MO:BOT	15.5
iPSC-derived midbrain organoids	manual	17.8
	MO:BOT	16.1

Figure 2: CellTiter-Glo® 3D viability assessment of MO:BOT– and manually generated 3D models. (**A**) Area-normalized ATP-based viability of HepG2 spheroids cultured on MO:BOT (purple) versus manual handling (yellow) (n=30). (**B–D**) Area-normalized viability of iPSC-derived kidney organoids (B, n=12)), cortical organoids (C, n=60), and midbrain organoids (D, n=14) under automated (purple) and manual (yellow) workflows, illustrating that the CellTiter-Glo® 3D assay can be applied uniformly across distinct 3D model types. Automation consistently increases mean viability in all four models. (**E**) Summary table reporting coefficient of variation (CV) for each model and handling method, demonstrating that MO:BOT reduces variability while producing higher and more consistent CellTiter-Glo® 3D viability signals across models. Data represent mean ± SD from approximately 12–48 spheroids or organoids per condition.

Discussion

Automation Enhances Viability and Data Consistency

Automation improves both cell viability and sample-to-sample consistency across 3D culture models, as demonstrated by up to 1.9-fold higher and less variable CellTiter-Glo® 3D signals compared to manual workflows. These gains persist after area-normalization and are consistent across diverse cell sources and formats, confirming that they reflect genuine biological improvements in culture quality rather than technical variability. These improvements likely arise from more consistent medium exchange, reduced handling stress

on fragile 3D structures, and precise control of incubation and assay timing, while the homogeneous, wash-free CellTiter-Glo® 3D format ensures that these biological improvements are captured without introducing additional variability from complex liquid-handling steps.

CellTiter-Glo® 3D as an Automation-Ready Endpoint Assay

CellTiter-Glo® 3D is particularly well suited for automated implementation because its single-addition, homogeneous protocol, 3D-optimized lytic capacity, and stable luminescent signal align directly with the strengths of robotic platforms. Direct reagent addition without washing simplifies programming and avoids spheroid or organoid loss, while the wide dynamic range and low background support robust viability quantification across different 3D model sizes and densities. In combination, the MO:BOT platform and CellTiter-Glo® 3D create an assay-centric workflow in which standardized handling yields 3D cultures with greater cell viability and the sensitive luminescent readout reliably quantifies these improvements.

Scalability, Throughput, and NAMs

The integration of CellTiter-Glo® 3D into the automated MO:BOT workflow has important has implications for scalability, throughput, and New Approach Methodologies (NAMs). By enabling parallel processing of multiple plates, unattended operation, and shareable standardized protocols, the MO:BOT platform overcomes key practical barriers that have limited the routine use of complex 3D models in larger studies. This combination of human-relevant and standardized spheroid and organoid systems with scalable, automation-ready viability assessment supports the broader adoption of NAMs aimed at reducing animal use while improving translational relevance in preclinical testing.

Conclusion

The integration of the CellTiter-Glo® 3D Cell Viability Assay into a fully automated MO:BOT workflow provides a robust solution for 3D spheroid and organoid viability assessment. The platform:

1. Automates cell seeding, medium exchange, image-based QC, and CellTiter-Glo® 3D sample preparation.

2. Increases viability signals by 1.3–1.9 fold across multiple 3D models compared to manual culture.
3. Reduces the coefficient of variation (CV) in viability measurements of organoid and spheroid cultures compared with manual handling.
4. Demonstrates that the homogeneous, single-addition CellTiter-Glo® 3D assay is well suited for quality control for complex 3D *in vitro* models and is compatible with automated platforms.

Together, MO:BOT and CellTiter-Glo® 3D enable scalable, standardized 3D culture workflows that are well aligned with emerging NAM and regulatory expectations for more human-relevant *in vitro* systems.

References

[1] Dutta, D., Heo, I., & Clevers, H. (2017). Disease modeling in stem cell-derived 3D organoid systems. Trends in Molecular Medicine, 23(5), 393–410. https://doi.org/10.1016/j.molmed.2017.02.007

[2] Jensen, C., & Teng, Y. (2020). Is it time to start transitioning from 2D to 3D cell culture? Frontiers in Molecular Biosciences, 7, 33. https://doi.org/10.3389/fmolb.2020.00033

[3] mo:re GmbH. (2025). Automating and standardizing organoid 3D cell culture workflows. https://more.science/organoid-3d-culture-automation/

[4] Hofer, M., & Lutolf, M. P. (2021). Engineering organoids. Nature Reviews Materials, 6(5), 402–420. https://doi.org/10.1038/s41578-021-00279-y

[5] Kim, J., Koo, B. K., & Knoblich, J. A. (2020). Human organoids: model systems for human biology and medicine. Nature Reviews Molecular Cell Biology, 21(10), 571–584. https://doi.org/10.1038/s41580-020-0259-3

[6] Promega Corporation. (2022). CellTiter-Glo® 3D Cell Viability Assay Technical Manual. https://www.promega.de/en/resources/protocols/technical-manuals/101/celltiter-glo-3d-cell-viability-assay-protocol/