

Cyto3D® Live-Dead Assay Kit



Versatile live/dead assay kit for 3D & 2D Culture, Organoids, Spheroids, Stem Cells, Fluorescence Microscopy



Versatile & Excellent for 3D/2D Cell Culture

Works with cells cultured in:

- VitroGel® hydrogels
- Animal-based ECM

Excellent for 3D cell culture.



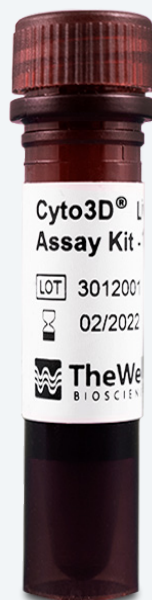
Even Staining & Clear Resolution Imaging

No unevenness and no background staining for clearer imaging.

**<10
MIN**

Fast & Cost-Effective

No pre-mixing required. Ready-to-use.
Fast one-step staining procedure for cell viability analysis.



Customer Testimonial



"When we used the Cyto3D® Live-Dead Assay Kit, we were able to obtain a very clear image without any unevenness in the well and almost no background staining on the gel."

We tried various reagents, but it was difficult because uneven staining occurred within or between the wells."

– from a Regenerative Medicine Company

The Cyto3D® Live-Dead Assay Kit is a **versatile live-dead assay kit for 3D and 2D Cell Culture, Organoids, Spheroids, Stem Cells, Fluorescence Microscopy**. It is used to determine the live/dead nucleated cells by using a quick one-step staining procedure for analysis on a dual-fluorescence system. The Cyto3D® Live-Dead Assay Kit is recommended for viability analysis of cells organoids cultured in 3D, 2D coating, and on monolayer and **works with cells cultured in animal-based ECMs and other hydrogel systems**.

The kit includes a premixed Acridine orange (AO) and propidium iodide (PI), both nuclear staining (nucleic acid binding). AO is permeable to both live and dead cells and stains all nucleated cells to generate green fluorescence. PI only penetrates the membranes of nucleated cells with compromised membranes and stains the dead cells to generate red fluorescence. Due to the quenching, when cells are stained with both AO and PI, all live nucleated cells fluoresce green, and all dead nucleated cells fluoresce red.

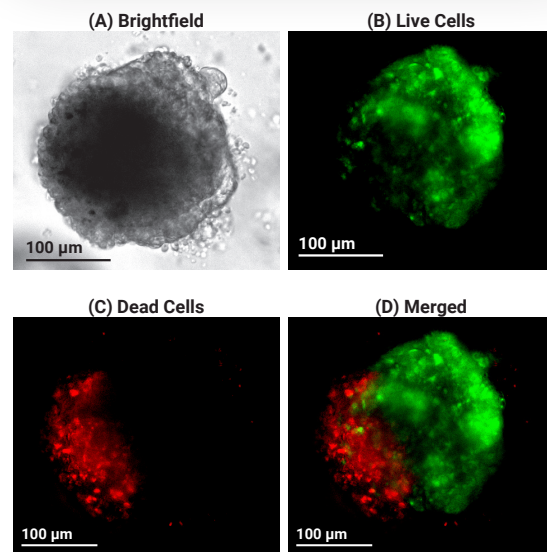


Figure 1: Live-dead cell viability images: Intestinal organoids stained with Cyto3D® Live-Dead Assay Kit. Intestinal organoids were cultured in regulated conditions for 5 days. Six microliters of Cyto3D® reagent was mixed with organoid culture media (each well includes 150 µL of organoid culture media and 150 µL of hydrogel volume). The mixture was incubated at 37 °C for 10-15 min, and the cells were observed under a fluorescence microscope. (A) A bright field image of a mature intestinal organoid. Images show live cells (B: Green) and dead cells (C: Red) present in a mature intestinal organoid.



1 Add Cyto3D® (2 µL).
No premixing required.

**5-15
MINUTES
Incubation**



2 Ready for detection.

Data and References

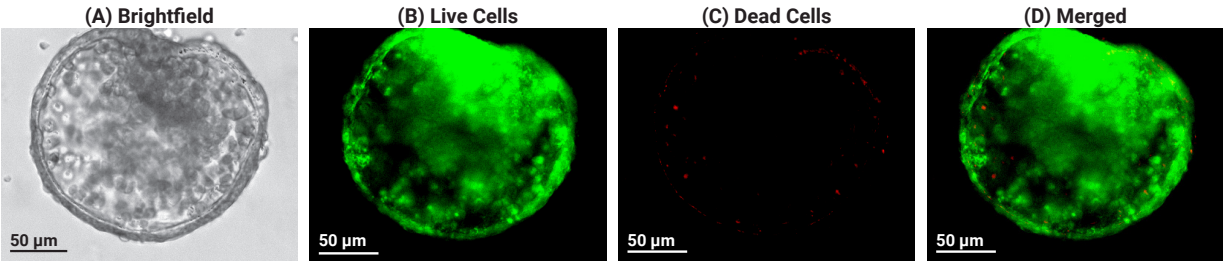


Figure 2: Live-dead cell viability images: Intestinal organoids stained with Cyto3D® Live-Dead Assay Kit. Intestinal organoids were cultured in regulated conditions for 2-3 days. Six microliters of Cyto3D® reagent was mixed with organoid culture media (each well includes 150 µL of organoid culture media and 150 µL of hydrogel volume). The mixture was incubated at 37 °C for 10-15 min, and the cells were observed under a fluorescence microscope. (A) A brightfield image of a young healthy intestinal organoid. Images show live cells (B: Green) and dead cells (C: Red) in a healthy intestinal organoid.

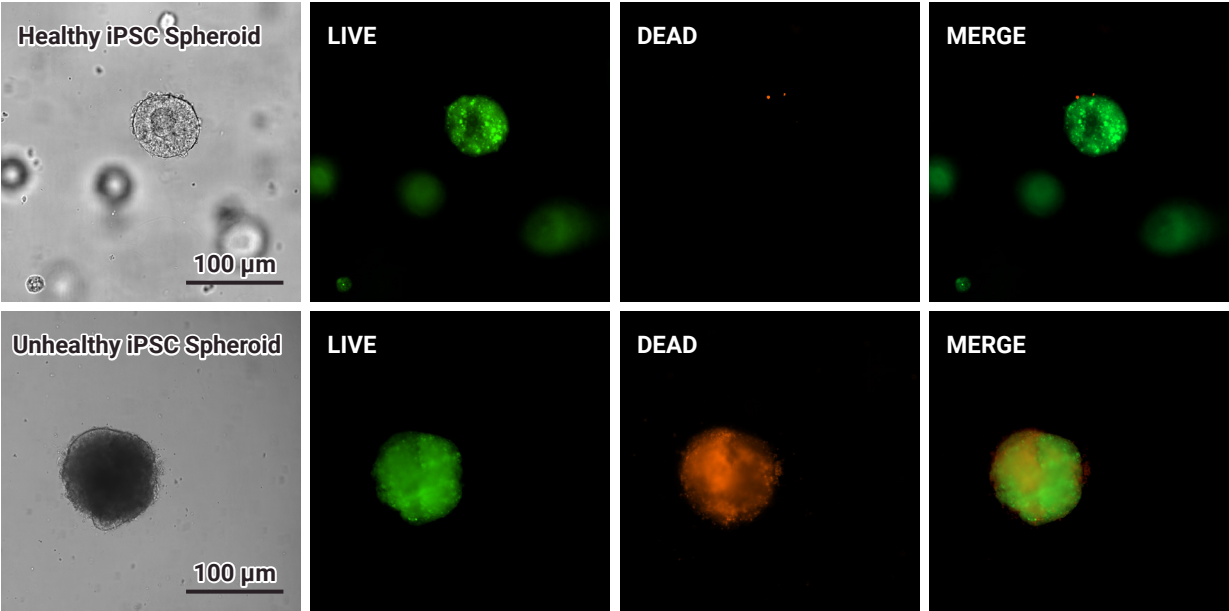




Figure 3: Live-dead cell viability images of stem cell spheroids. Stem cells were static suspension-cultured in VitroGel® STEM (Cat. #: VHM02) for 5 days. Two microliters (2 µL) of Cyto3D® Live-Dead Assay reagent was added to each well containing 100 µL cell suspension. The mixture was incubated at 37 °C for 5-10 min. The cells were then observed under a fluorescent microscope. The images show the Live (green) and Dead (orange) stem cell spheroids cultured in a 3D hydrogel matrix. The live-dead dyes of Cyto3D® Live-Dead Assay Kit can successfully penetrate into large cell spheroids for cell viability analysis.

References / Publications

- Kim, M. S., & Kim, M. S. (2025). **Deubiquitination of epidermal growth factor receptor by ubiquitin-specific peptidase 54 enhances drug sensitivity to gefitinib in gefitinib-resistant non-small cell lung cancer cells.** PLoS ONE, 20(4), e0320668–e0320668. <https://doi.org/10.1371/journal.pone.0320668>
- Chang, W.-H., Chin, A. I., & Chen, C.-H. (2025). **Protocol for a patient-derived preclinical platform to model tumor-immune interactions and evaluate therapeutic efficacy.** STAR Protocols, 6(1), 103623–103623. <https://doi.org/10.1016/j.xpro.2025.103623>
- Donato, M. D., Cristiani, C. M., Capone, M., Garofalo, C., Madonna, G., Passacatini, L. C., Ottaviano, M., Paolo Antonio Ascierto, Auricchio, F., Carbone, E., Migliaccio, A., & Castoria, G. (2025). **Role of the androgen receptor in melanoma aggressiveness.** Cell Death and Disease, 16(1). <https://doi.org/10.1038/s41419-025-07350-4>
- Kawai-Kawachi, A., Lenormand, M. M., Astier, C., Herbel, N., Cutrona, M. B., Ngo, C., Garrido, M., Eychenne, T., Dorvault, N., Bordelet, L., Song, F. F., Bouyakoub, R., Loktev, A., Romo-Morales, A., Henon, C., Colmet-Daage, L., Vibert, J., Drac, M., Brough, R., & Schwob, E. (2025). **Replication Stress is an Actionable Genetic Vulnerability in Desmoplastic Small Round Cell Tumors.** Cancer Res. <https://doi.org/10.1158/0008-5472.CAN-23-3603>
- Kim, K., Lee, Y., Jung, K. B., Kim, Y., Jang, E., Lee, M., Son, M., & Lee, H. J. (2024). **Highly Stretchable 3D Microelectrode Array for Noninvasive Functional Evaluation of Cardiac Spheroids and Midbrain Organoids.** Advanced Materials. <https://doi.org/10.1002/adma.202412953>



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Cyto3D® Live-Dead Assay Kit:
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kit-cyto3d/](http://www.thewellbio.com/product/live-dead-cell-assay-kit-cyto3d/)



Read the white paper on **A Quick Organoid Viability by using Cyto3D® Live-Dead Assay Kit**
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Product	Cat No.	Size
Cyto3D® Live-Dead Assay Kit	BM01	1 mL