

## 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 **hydrogels** and **animal-based ECM**. Excellent for 3D cell culture.



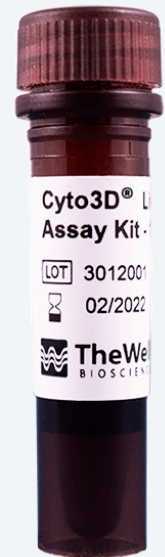
### Even Staining & Clear Resolution Imaging

**No unevenness** and **no background staining** for clearer imaging.



### Fast & Cost-Effective

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

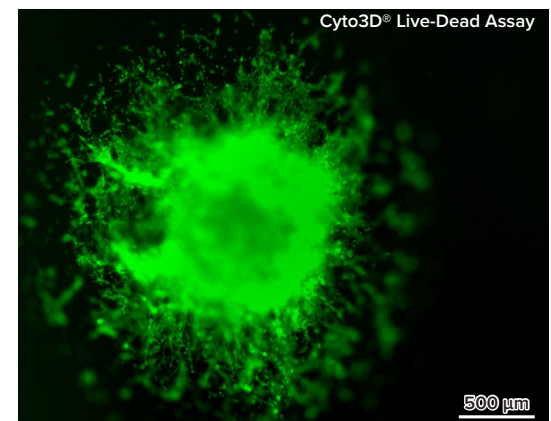


The **Cyto3D® Live-Dead Assay Kit** is a **versatile live-dead assay kit for 3D and 2D Cell Culture, Organoids, Spheroids, Stem Cells, Fluorescence Microscopy**. It is used to determine the live/dead nucleated cells by using a quick one-step staining procedure for analysis on a dual-fluorescence system. 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.



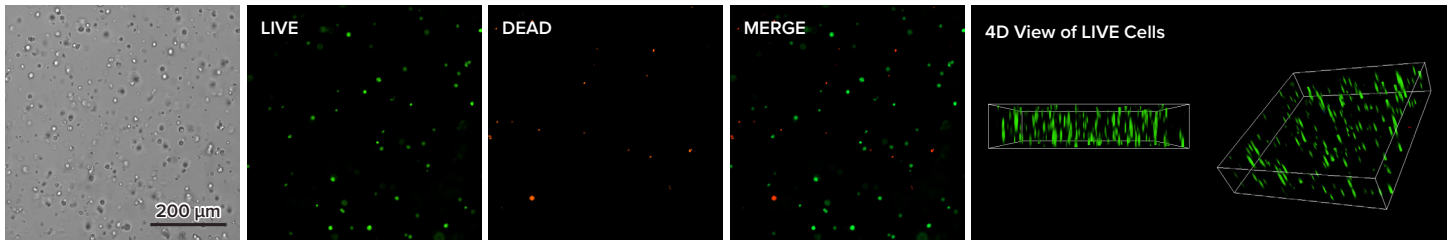
## Data and References



**Figure 1. Live-dead assay of 3D spheroid U-87 MG cells cultured in VitroGel® Hydrogel Matrix.**

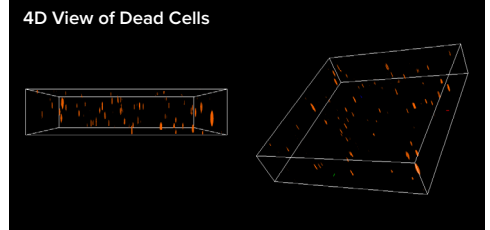
The live-dead assay of day-7 U-87 MG cells in VitroGel® Hydrogel Matrix revealed significant cell viability when cultured in this biofunctional hydrogel for 3D spheroid invasion assay.

Source: *3D Spheroid Invasion Assay With the Xeno-free, Bio-Functional VitroGel® Hydrogel Matrix - TheWell Bioscience*



**Figure 2. Live-dead cell viability analysis by using Cyto3D® Live-Dead Assay Kit.**

Glioblastoma cells (SF 298, about 60% cell viability) were 3D cultured in VitroGel® system for 2 days. 2 µL of Cyto3D® Live-Dead Assay reagent was added to each well containing 50 µL hydrogel and 50 µL cover medium. 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) cells within the 3D hydrogel matrix. The z-stack images of cells within hydrogel were then 3D reconstructed and shown in the 4D view images. The live and dead cells at higher levels of the hydrogel are clearly shown in the images by using Cyto3D® Live-Dead Assay Kit.



**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. 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

- Wang, H., Zhang, Y., Miao, H., Xu, T., Nie, X., & Cheng, W. (2024). **CircRAD23B promotes proliferation and carboplatin resistance in ovarian cancer cell lines and organoids.** *Cancer Cell International*, 24(1). <https://doi.org/10.1186/s12935-024-03228-1>
- Miao, H., Meng, H., Zhang, Y., Chen, T., Zhang, L., & Cheng, W. (2024). **FSP1 inhibition enhances olaparib sensitivity in BRCA-proficient ovarian cancer patients via a nonferroptosis mechanism.** *Cell Death & Differentiation*, 1–14. <https://doi.org/10.1038/s41418-024-01263-z>
- Babl, N., Hofbauer, J., Matos, C., Voll, F., Ayse Nur Menevse, Rechenmacher, M., Mair, R., Philipp Beckhove, Herr, W., Siska, P. J., Renner, K., Kreutz, M., & Schnell, A. (2023). **Low-density lipoprotein balances T cell metabolism and enhances response to anti-PD-1 blockade in a HCT116 spheroid model.** *Frontiers in Oncology*, 13. <https://doi.org/10.3389/fonc.2023.1107484>
- Belén, A., Sacconi, A., Tremante, E., Lulli, V., Caprara, V., Rosanò, L., Goeman, F., Carosi, M., Marta Di Giuliani, Vari, G., Silvani, A., Pollo, B., Garufi, C., Ramponi, S., Simonetti, G., Ciusani, E., Chiara Mandoj, Stefano Scabini, Villani, V., & Agnese Pò. (2023). **A diagnostic circulating miRNA signature as orchestrator of cell invasion via TKS4/TKS5/EFHD2 modulation in human gliomas.** *Journal of Experimental & Clinical Cancer Research*, 42(1). <https://doi.org/10.1186/s13046-023-02639-8>

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



Read the full application note about 3D Spheroid Invasion Assay With the Xeno-free, Bio-Functional VitroGel® Hydrogel Matrix:  
[www.thewellbio.com/application-notes/3d-  
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 hydrogel-matrix/](http://www.thewellbio.com/application-notes/3d-spheroid-invasion-assay-xeno-free-vitro-gel-hydrogel-matrix/)



Product	Cat No.	Size
Cyto3D® Live-Dead Assay Kit	BM01	1 mL