TheWell BIOSCIENCE

3D Cell Culture and Beyond

Cyto3D® Live-Dead Assay Kit

Versatile, live/dead cell viability analysis for 3D and 2D cell culture

Catalog #: BM01
Protocol and Case Studies

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For research use only. Not intended for use in diagnostics or therapeutic procedures.

Cyto3D® Live-Dead Assay Kit

Protocol for Cell Viability Analysis

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MATERIALS

- **Cells cultured in VitroGel® system**
- **Cyto3D® Live-Dead Assay Kit (Catalog #: BM01)**
- **Micropipette; Low retention pipette tips**
- **Fluorescence microscope, flow cytometer, microplate reader or fluorescence cell counter**

PROTOCOL

1. Bring the Cyto3D® Live-Dead Assay Kit to room temperature.
2. Add 2 µL of Cyto3D® reagent to every 100 µL total volume in a well.

   Note: Adjust the volume of Cyto3D® reagent according to the total volume of hydrogel and medium.

   For example, for 3D cell culture, 50 µL hydrogel + 50 µL cover medium = total volume of 100 µL.
3. Incubate the cells at 37°C for 5-10 minutes. The cells are ready for cell viability detection.

   Note: For organoids, incubate at 37°C for 10-15 minutes.

1 Add Cyto3D®. No premixing required.

2 Ready for detection.

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CASE STUDY 1

Organoid Viability Measurement by Using Cyto3D® Live-Dead Assay Kit

Materials

- **Specimens:** Intestinal Organoids
- **Hydrogel:** VitroGel® ORGANOID-3 (Catalog #: VHM04-3) and Matrigel® Matrix
- **Biomarker:** Cyto3D® Live-Dead Assay Kit (Catalog #: BM01)
- **Instrument:** Fluorescence Microscope

Intestinal organoids were thawed and suspended in both VitroGel® and Matrigel®. For VitroGel®, a suspension of organoids was prepared with 3X concentration of critical growth factors. VitroGel® ORGANOID-3 and organoid suspension were mixed at a 2:1 v/v ratio and kept for 15 minutes at room temperature for a soft gel formation. Similarly, for gel formation, organoids suspended in Matrigel® were incubated for 15 minutes at 37°C. Once ready, organoids were treated with 1X organoids culture medium supplemented with essential growth factors/inhibitors to stimulate organoid growth. Two sets of intestinal organoids were grown in 3D for 2 days and 5 days, respectively, in 5% CO_2 and at 37°C supplemented with essential growth factors/inhibitors to stimulate organoid growth. Two sets of intestinal organoids were grown in 3D for 2 days and 5 days, respectively, in 5% CO_2 and at 37°C.

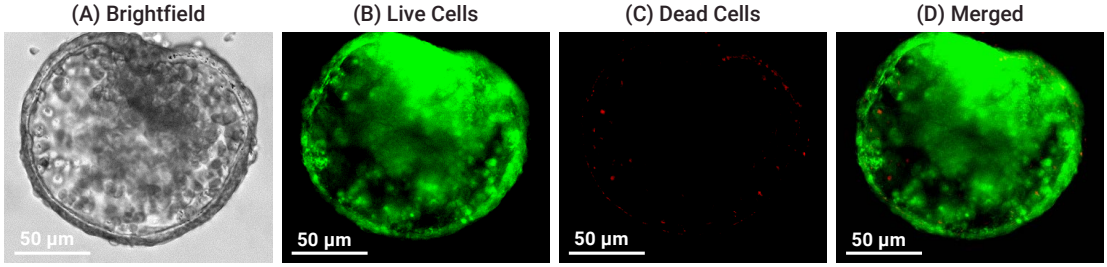

Figure 1: Live-dead cell viability images: Intestinal organoids stained with Cyto3D® Live-Dead Assay Kit.
Intestinal organoids were cultured in regulated conditions for 2-3 days. Six microliters (6 µL) of Cyto3D® Live-Dead Assay reagent were mixed with organoid culture media (each well includes 150 µL 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.

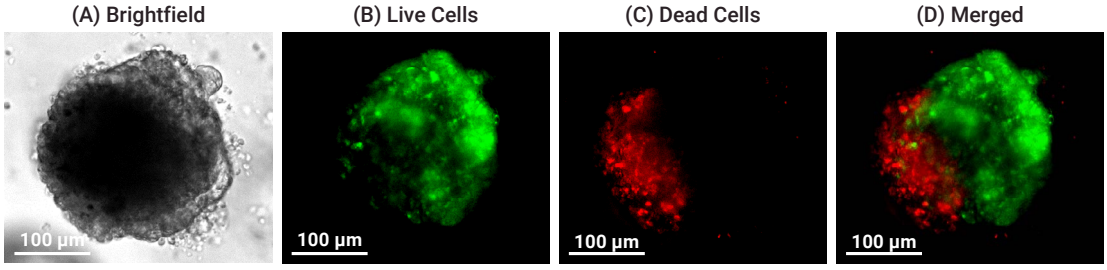

Figure 2: Live-dead cell viability images: Intestinal organoids stained with Cyto3D® Live-Dead Assay Kit.
Intestinal organoids were cultured in regulated conditions for 5 days. Six microliters (6 µL) of Cyto3D® reagent were mixed with organoid culture media (each well includes 150 µL of organoid culture 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) in a mature intestinal organoid.

CASE STUDY 2

Live-dead cell viability analysis of Glioblastoma cells using Cyto3D® Live-Dead Assay Kit

Materials

- **Specimens:** Glioblastoma cells
- **Hydrogel:** VitroGel® hydrogel
- **Biomarker:** Cyto3D® Live-Dead Assay Kit (Catalog #: BM01)
- **Instrument:** Fluorescence Microscope

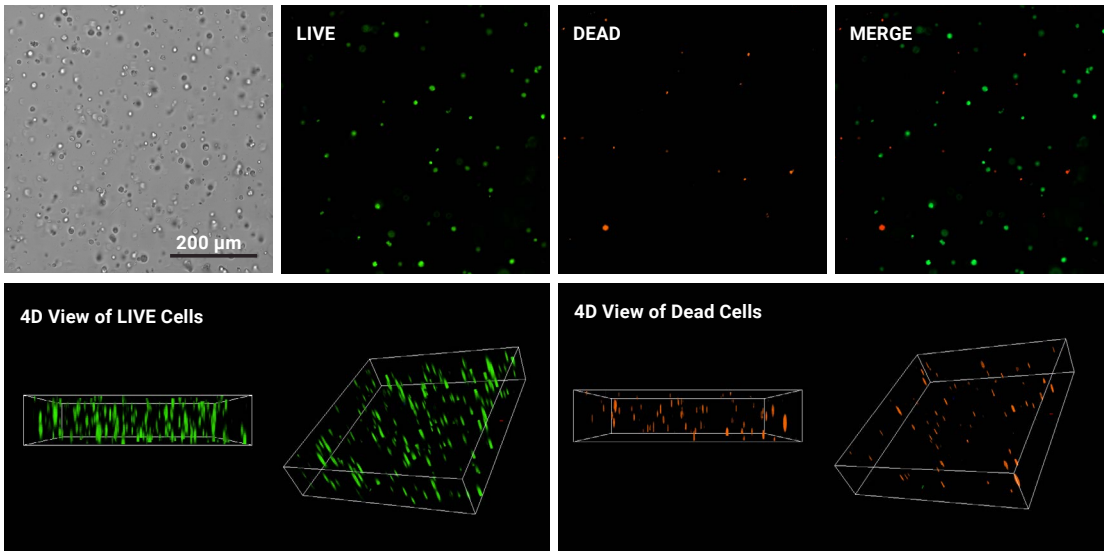

Figure 3. Live-dead cell viability analysis by using Cyto3D® Live-Dead Assay Kit.
Glioblastoma cells (SF 298, about 60% cell viability) were 3D cultured in VitroGel® system for 2 days. Two microliters (2 µL) of Cyto3D® Live-Dead Assay reagent was added to each well containing 50 µL hydrogel and 50 µL cover medium. The mixture was incubated at 37°C for 5-10 min. The cells were then observed under a fluorescence 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 using the Cyto3D® Live-Dead Assay Kit.

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