TheWell BIOSCIENCE

Product Data Sheet

Revision v1.02

Cyto3D® Live-Dead Assay Kit

Catalog Numbers:
BM01

Usage restrictions: For Research Use Only. Not For Use In Diagnostic Procedures.

Product Description

The Cyto3D® Live-Dead assay kit is used to determine the live/dead nucleated cells by using a fast one-step staining procedure for analysis on a dual-fluorescence system. This kit is recommended for viability analysis of cells cultured in 3D, 2D coating and on monolayer.

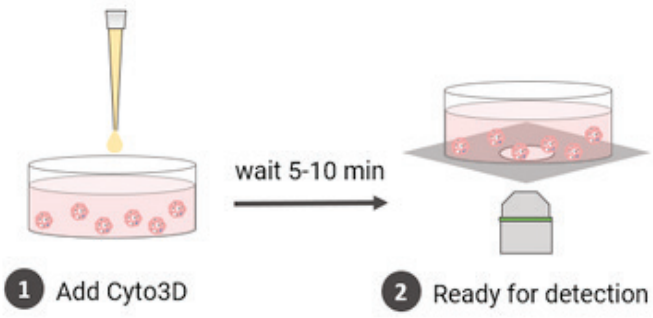
- Ready-to-use
- Fast
- Sensitive
- Excellent for 3D cell cultures
- Cost-effective

Acridine orange (AO) and propidium iodide (PI), both nuclear staining (nucleic acid binding) dyes, are used in this kit. 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 (the PI reduces the fluorescence intensity of the AO by fluorescence resonance energy transfer (FRET)). Non-nucleated materials such as red blood cells, platelets and debris do not fluorescence and are ignored by fluorescence microscopes.

Dual-Fluorescence Viability, using AO and PI, is the recommended viability analysis method for cell lines, primary cells, and stem cells.

SPECIFICATIONS	
Formulation	Premixed acridine orange (AO) and propidium iodide (PI) nuclear staining dyes
Use	Live dead cell viability analysis for 3D and 2D cell culture
Detection Method	Fluorescent
Excitation/Emission	AO (494/517nm), PI (535/617nm)
Standard Filters	AO (GFP), PI (Texas Red)
For use with (Equipments):	Fluorescence microscope, flow cytometer, microplate reader, fluorescence cell counter.
Storage	2 to 8 °C (Protect from light) Ships at ambient temperature
Stability	24 months from date of manufacture
Sizes	1 mL
Uses	500 test (at 2 µL per 100 µL)

Easy setup and use



Protocol

More protocols can be found at www.thewellbio.com/protocols

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 = 100 µL total volume).
3. Incubate the cells at 37 °C for 5-10 minutes. The cells are ready for cell viability detection.

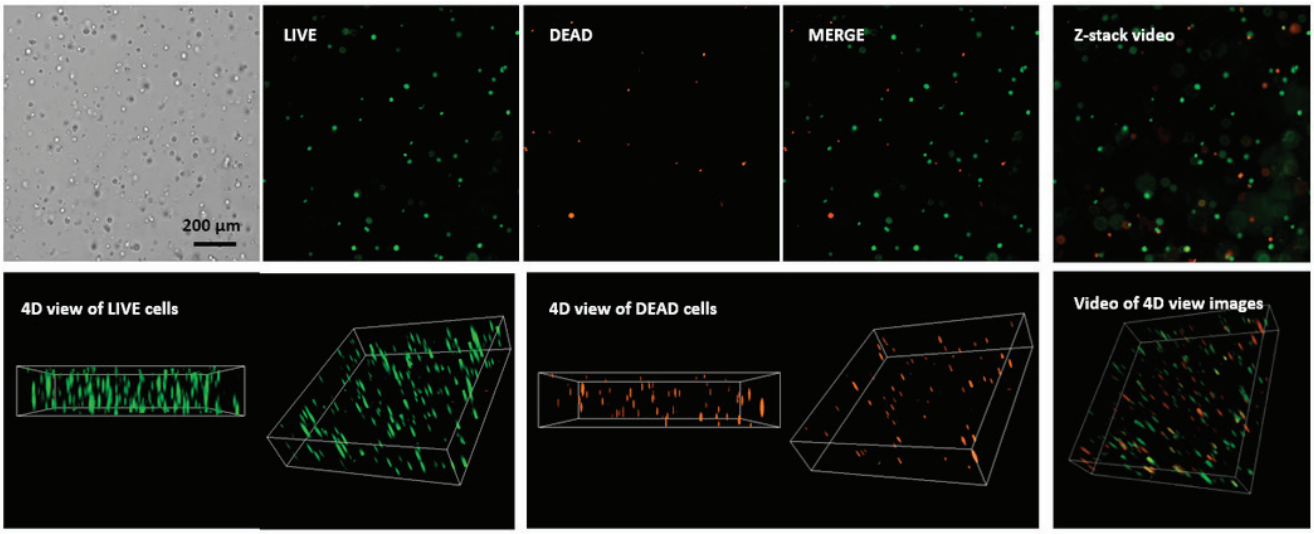


Figure 1. Live-dead cell viability analysis by using Cyto3D Live-Dead Assay Kit.
Glioblastoma cells (SF 298, about 60% cell viability) were 3D cultured in VitroGel system for 2 days. 2 µL of Cyto3D reagent was added to each well containing 50 µL hydrogel and 50 µL cover medium. The mixture was incubated 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 showed in the 4D view images. The live and dead cells at higher levels of the hydrogel clearly show up in the images by using Cyto3D Live-Dead Assay Kit.

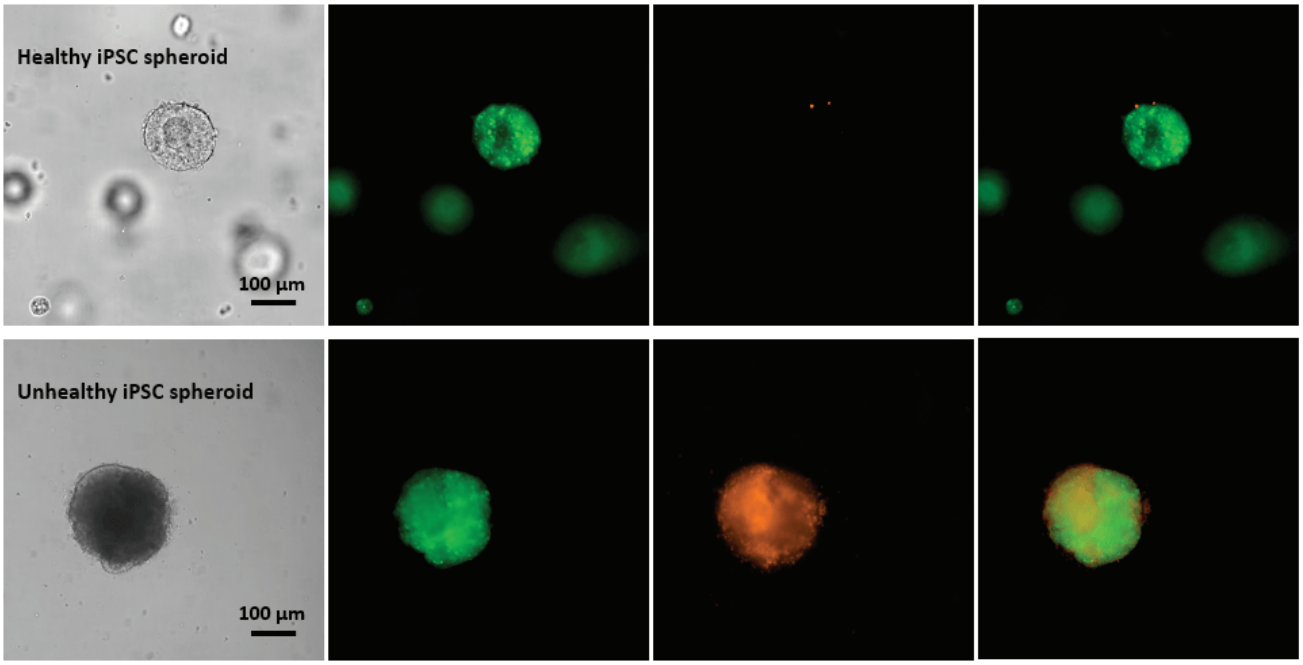


Figure 2. Live-dead cell viability images of stem cell spheroids.
Stem cells were static suspension culture in VitroGel STEM (cat# VHM02) for 5 days. 2 µL of Cyto3D reagent was added to each well containing 100 µL cell suspension. The mixture was incubated at 37 °C for 5-10 min. The cells were then observed 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 the big cell spheroids for cell viability analysis.

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