Live-Dead Cell Viability Assay Kit

For 3D and 2D Cell Culture

Cat. # CBA415

pack size: 1 kit

Store at -20ºC

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.
NOT FOR HUMAN OR ANIMAL CONSUMPTION.

Data Sheet

Background

The Live-Dead Cell Viability Assay Kit is a quick and simple three-color assay to measure cell viability. The kit consists of Calcein-AM (stains live cells), Propidium Iodide (stains dead cells) and Hoechst 33342 (stains all cells). The kit has been optimized for 3D cell culture (spheroids, human organoids and 3D matrices) and 2D cell culture (multiple cell types). The kit can be used for flow cytometry, fluorescence microscopy and with fluorescence microplate readers. The kit determines viability of cells based on plasma membrane integrity and esterase activity.

Advantages:

1. Tested and optimized for 3D cell culture, Spheroid culture, organoid culture, 2D cell culture and flow cytometry.
2. Easy to use and quick protocol.
3. Stronger intensity of Calcein AM and Hoechst dyes compared to other products.

Kit Components

Vial 1: Green cap: Calcein AM in DMSO
Vial 2: Propidium Iodide in DMSO
Vial 3: Hoechst 33342 in DMSO

Storage

Store in freezer at -20°C and protect from light. Allow the reagents to warm to room temperature and centrifuge each vial briefly to collect contents at bottom of vial before opening. The unused reagents can be refreezed at -20°C.

Spectral Properties

Calcein AM: Ex/Em: 490/515 nm
Propidium Iodide: Ex/Em: 535/617 nm
Hoechst 33342: Ex/Em: 361/486 nm

Protocol for 2D Culture

Preparation of Adherent cells

1. Plate NIH3T3 cells in an appropriate cell culture medium using a regular flat, clear bottom 8-well and 400-500 µl medium per well.
2. For adherent cells, cell confluence at time point of treatment should not be more than 50%.
3. Induce cytotoxicity (e.g., by adding a cytotoxic compound in a dose-dependent manner), and then stain cells using the instructions below.

Reagent Preparation and staining

1. Allow all three vials of reagents to equilibrate to room temperature. Before opening the vials, centrifuge for 15 seconds in a microcentrifuge to collect the solution to the bottom of the vial.
2. Mix 12 ml of cell culture medium and 12 ml of PBS in 1:1 ratio. To this mixture add 5 µl of Calcein AM (vial 1, green cap), 20 µl of Propidium iodide (vial 2, red cap) and 8 µl of Hoechst 33342 (vial 3, blue cap) to prepare 24 ml of dye solution.
3. Vortex the dye solution to thoroughly mix the components.
4. Aspirate the culture medium from the 8-well plate and add 400-500 µl of dye mixture to each well.
5. Incubate the plate for 30 min at 37ºC.
6. Analyze the plate for cell count and viability using a suitable fluorescence microscope with the appropriate excitation and emission filters, or else use an appropriate system for automated image acquisition and analysis. See figure 1 for live-dead staining of a 2D culture of NIH3T3 cells.

Protocol for Spheroid and Organoid Culture

Preparation of HepG2 spheroids

1. Culture HepG2 cells in monolayer until 90% confluent. On day 1, harvest the cells using trypsin and resuspend HepG2 cells in DMEM medium with 10% FBS at 0.4 x 10^6 cells/ml.
2. Mix the cell supernatant with ice-cold Corning® Matrigel hESC-qualified matrix in a 1:19 (v/v) ratio.
3. Aliquot 50 µl the cell-Matrigel mixture to 8-well confocal chamber slide (Lab-TEK #155409). After the gelation, add 500 µl DMEM 10% FBS medium. Change medium every other day.
4. On day 10, induce cytotoxicity or treat the spheroid with test compounds as planned.

Preparation of Human colon organoids

Human iPSC-derived colon organoids were generated using in-house developed protocols in 25 µl dome in 24 well plate.

Reagent Preparation and staining

1. Mix 6 ml of cell culture medium and 6 ml of PBS in a 1:1 ratio. To this mixture add 5 µl of Calcein AM (vial 1, green cap), 20 µl of Propidium iodide (vial 2, red cap) and 8 µl of Hoechst 33342 (vial 3, blue cap) to prepare 12 ml of dye solution.
2. Vortex the dye solution to thoroughly mix the components.
3. Aspirate the cell culture medium from the culture plate and add appropriate volume of dye mixture to each well.
4. Incubate the plate for 60 min at 37ºC.
5. Analyze the plate for cell count and viability using a suitable fluorescence microscope with the appropriate excitation and emission filters, or else use an appropriate system for automated image acquisition and analysis. See figure 2 for live-dead staining of HepG2 spheroids and figure 3 for live-dead staining of hiPSC-derived colon organoids.

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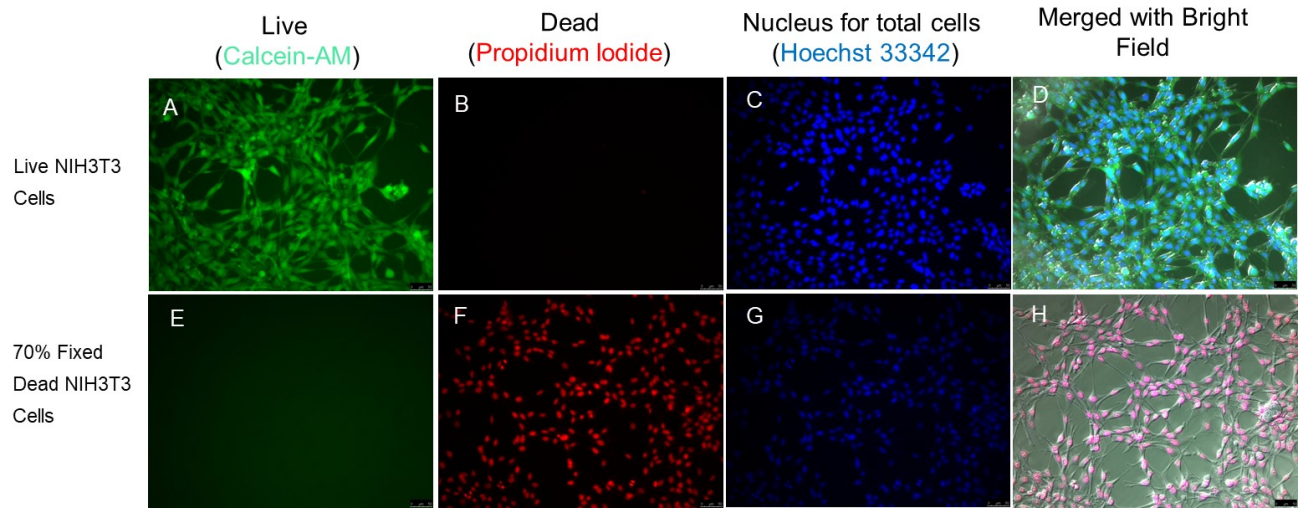

Figure 1. NIH3TS cells were cultures in an 8-well chamber slide and stained with live-dead cell viability assay kit. The top row shows live culture of cells stained with Calcein-AM (A), propidium iodide (B), Hoechst 33342 (C) and merged brightfield image (D). The lower panel of images (E-H) shows cells fixed with 70% ethanol and then stained with the kit for Calcein-AM (E), propidium iodide (F), Hoechst 33342 (G) and merged brightfield image (H).

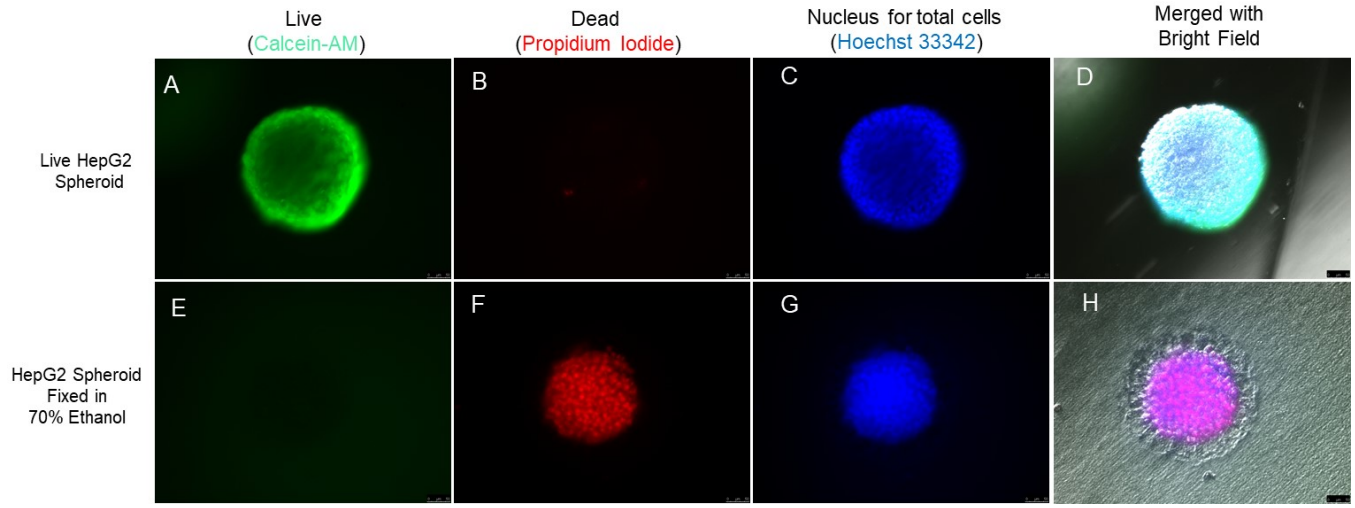

Figure 2. HepG2 spheroids were cultured in an 8-well chamber slide and stained with the live-dead assay kit. The top row shows live cell spheroid culture stained with Calcein-AM (A), propidium iodide (B), Hoechst 33342 (C) and merged brightfield image (D). The lower panel of images (E-H) shows cells fixed with 70% ethanol and then stained with Calcein-AM (E), propidium iodide (F), Hoechst 33342 (G) and merged brightfield image (H).

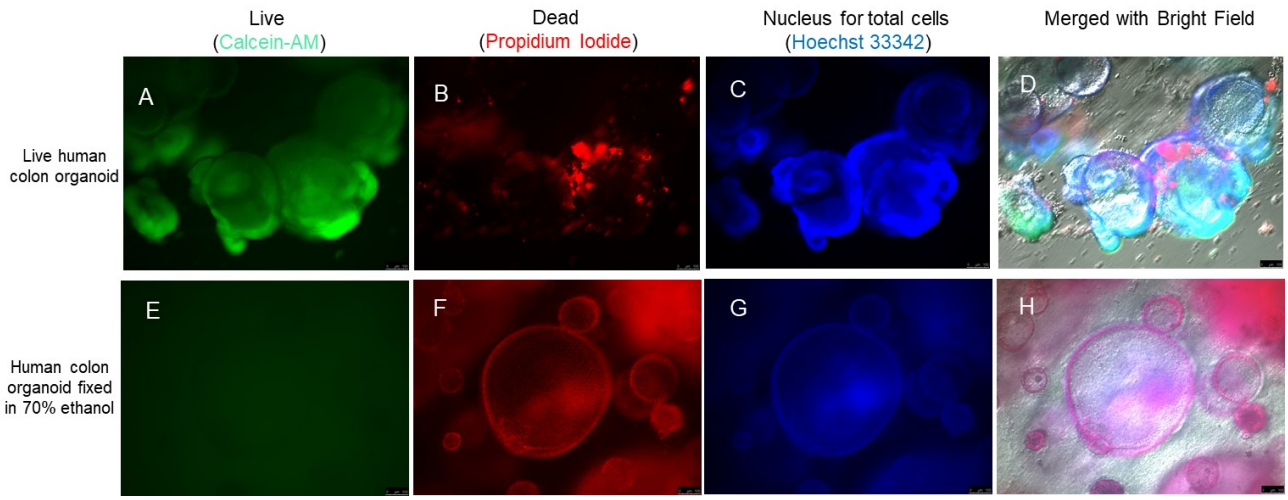

Figure 3. Human iPSC-derived colon organoids were cultured in 25 µl domes in 24-well plates and stained with live-dead cell viability assay kit. The top row shows live cultures stained with Calcein-AM (A), propidium iodide (B), Hoechst 33342 (C) and merged brightfield image (D). The lower panel of images (E-H) shows organoids fixed with 70% ethanol and then stained with Calcein-AM (E), propidium iodide (F), Hoechst 33342 (G) and merged brightfield image (H).