RASTRUM

Protocol

In Situ Viability Analysis of 3D Cell Models

Document Number | WET.BCM.0007.PRO_v1.03
Date of issue | January 2021

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Introduction

This protocol outlines a method to assess viability of 3D cell models created with RASTRUM using fluorescent dyes for distinguishing live and dead cells via fluorescence microscopy. The esterase substrate calcein AM stains live cells green, while the membrane-impermeable DNA dye Ethidium homodimer III (EthD-III) stains dead cells red. Hoechst 33342 nucleic acid stain is cell-permeable and emits blue fluorescence when bound to dsDNA in live and dead cells.

Equipment and reagents required, not provided

- Fluorescence microscope for red (ex ~600nm), green (ex ~488nm), and blue (ex ~350nm) light
- Viability/Cytotoxicity Assay Kit for Animal Live & Dead Cells (Biotium, cat #30002)
- 10 µg/mL Hoechst 33342 (ThermoFisher, cat #H3570)
- 1 x Phosphate Buffered Saline (PBS)

Protocol

1. Prepare working solutions of 1 µM calcein AM, 2 µM EthD-III, and 10 µg/mL Hoechst 33342 in PBS. Protect from light where possible.
2. From each well, completely remove cell culture medium and discard.
3. Wash each well by gently adding 200 µL PBS. Remove PBS and discard.
4. Add 100 µL working solution to each 3D cell model.
5. Incubate well plate at 37°C, 30 minutes.
6. Remove stain solution and wash each well by gently adding 200 µL PBS. Remove PBS and discard.
7. Add 100 µL PBS to each well for imaging.
8. Using a fluorescence microscope, acquire fluorescence images and calculate % calcein AM positive (live) and EthD-III positive (dead) cells relative to Hoechst positive nuclei.

Note: We recommend using an image analysis tool to calculate % live/dead such as CellProfiler (Broad Institute).

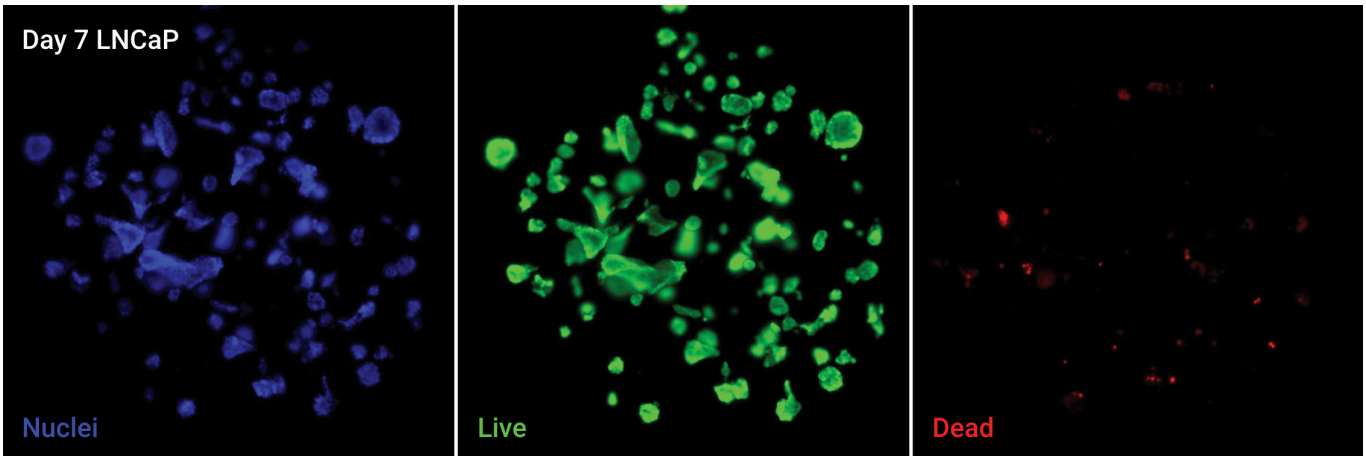

Figure 1: LNCaP 3D cell models stained with calcein AM, EthD-III, and Hoechst 33342

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