RASTRUM

Protocol | *In situ* viability analysis of printed 3D cell models

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Introduction

This protocol outlines a method to calculate viability of 3D cell models created with RASTRUM using two 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 but not provided

- Fluorescence microscope for red (ex ~600nm), green (ex ~488nm), and blue (ex ~305nm) light
- Viability/Cytotoxicity Assay Kit for Animal Live & Dead Cells (Biotium, cat #30002)
- 5 µg/mL Hoechst 33342 (Thermo, cat #H3570)

Protocol

1. Prepare working solutions of calcein AM, EthD-III, and Hoechst 33342 according to the manufacturer's guidelines.

Note: Working solutions should be diluted in PBS and should be protected from light exposure where possible.

2. From each well, completely remove cell culture medium and discard.
3. Wash each well by gently adding 200µL PBS. Remove PBS and discard.
4. Add 100µL stain 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 200µL PBS. Remove PBS and discard.
7. Add 100µL 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).

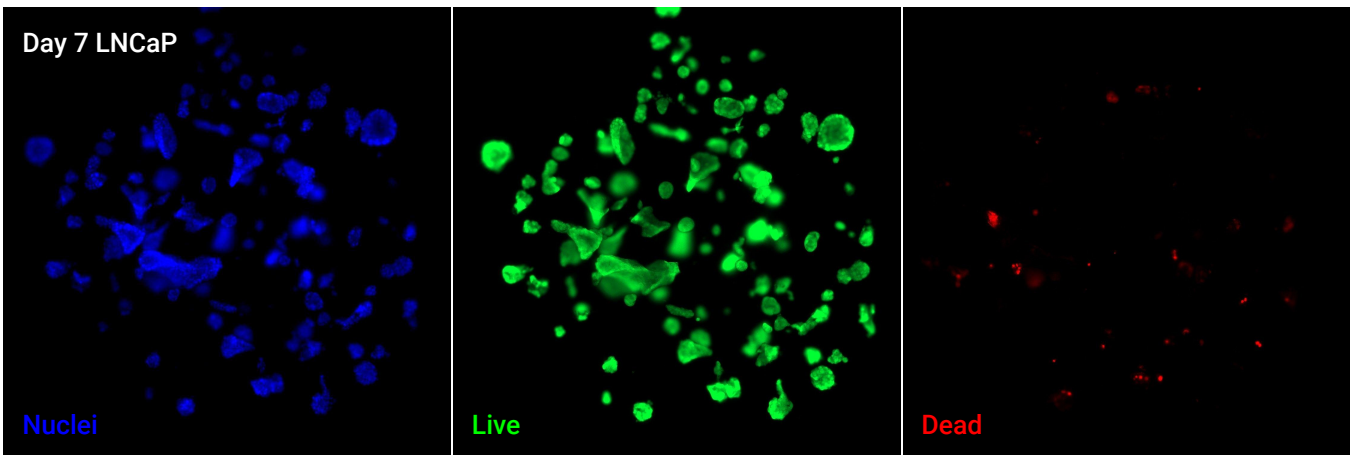

Figure 1. LNCaP 3D cell models stained with calcein AM, EthD-III, and Hoechst 33342