Monitoring cell health in real-time / time-lapse cell-based assays

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Introduction

The purpose here is to validate a novel far-red DNA binding viability probe, DRAQ7™, in real-time cell-based assays for 2-D and 3-D models. This requires DRAQ7™ to neither synergize nor interfere with toxicants / anti-cancer compounds. It must be capable of addition at any stage of an assay to permit real-time monitoring of membrane integrity loss, retaining its functionality throughout.

As background, DRAQ7™’s DNA-specificity allows monitoring cell-by-cell while spectral properties permit incorporation in multi-colour experiments e.g. with vital probes, mitochondrial health probes or Annexin V. DRAQ7™ has previously been shown to be truly cell-impermeant yet retains DNA binding and far-red fluorescence of the DRAQ chromatophore which makes it compatible with HCS imagers, fluorescence microscopes and cytometers. The excitation/emission properties are particularly suited to penetrative imaging of multi-cellular structures and thick ex-plant tissues, whilst limiting risks of short wavelength DNA damage.

DRAQ7™ - Identity

- pure synthetic DNA-specific anthraquinone probe
- supplied in aqueous, ready-to-use, no DMSO
- ship ambient, stores at 2-8°C
- excitation: red - low photo-toxicity / DNA damage
- emission: far-red (>675 nm) - “DRAQ” chromatophore
- compatible with culture medium, buffers
- low photobleach, chemically stable, no-wash

Core Performance – Viability Monitoring

As shown in figures 3 & 4, DRAQ7™ demonstrates the fundamental requirement of a viability or cell health reporter in both flow cytometric and image-based assays.

Real-Time / Long-Term Cell Health Monitoring

DRAQ7™ at both excess or standard concentration shows no impact on cell growth compared to untreated controls making DRAQ7™ an ideal read-out for cell death in real-time viability and toxicity assays (Figure 9).

Cell-by-Cell and Spatio-Temporal Reporting

DRAQ7™ utilises the DRAQ chromatophore, a DNA intercalator (figure 5a), evidenced by equal performance as a counterstain in fixed cell imaging vs. DRAQ5™ (figure 5b).

Spectral (and Cross-Platform) Compatibility

DRAQ7™ is optimally red excited (fig.1) emitting in the far-red / NIR (fig. 2). Spectral compatibility with a broad range of vis. range floors is described in figure 7. Utility in imaging is shown in figure 8, showing DRAQ7™ with supravital stains and fluorescein-activated GPCR ligands.

3D Imaging of Cell Health (in real-time)

Based on this accumulated data Imagen-Therapeutics have utilised DRAQ7™ to monitor cell viability in real-time 2-D and 3-D spheroid/micro-tissue assays including a study on glioblastoma (GBM)-derived stem cell lines in response to a library of chemotherapeutic agents (as shown in figure 10, Thermo Arrayscan; detailed elsewhere).

Real-time Cell Health – HCS Case Study

To explore this further a HCS screen was established with Imagen Therapeutics on Nexclom Bioscences’ Celigo 5.

Study: test a panel of 56 compounds on MCF-7 cell viability, 500 cells/well were plated in 20 μM DMEM at Day -2 in a 384-well tissue culture plate. Medium was replenished and compounds were added on day 0. Plates were read on the Celigo 5 using the Dead + Total Viability application on Day 1, 2 and 3. Total cells were identified via the brightfield channel and dead cells via the Far-Red channel using DRAQ7™.

Discussion / Future Work

Real-time or time-lapse cell health monitoring offers a new paradigm for cell biology and drug development. The performance characteristics of DRAQ7™ make it an ideal choice for this, and importantly it is amenable to automation.

Future investigations may include the testing of DRAQ7™ in more complex tissue models, embryogenesis and potentially in vivo.

References