Image Acquisition for Cell Health and Morphometric Assay using the InCell Analyser 6000

SOP number: WP1/011

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Developed under NC3R project: NC/C013203/1
INTRODUCTION

The protocol describes the image acquisition and analysis procedure for Cell Health and Morphometric Assay. The plate is scanned using automated imaging platform InCell Analyser 6000 (GE Healthcare,UK), which is equipped with 4 channels: DAPI, FITC, dsRED and Cy5. The nucleus is identified by Hoechst 33342 staining in the DAPI channel (blue staining). Cell membrane permeability is assessed by determining signal intensity in the nucleus in the FITC channel (green staining). Mitochondrial toxicity is measured by the signal decrease in the dsRED channel (orange staining). Cell plasma is labelled by Cell Mask stain in the Cy5 channel (red staining).

Approximate fluorescence excitation/emission maxima: Hoechst 33342: 350/461 nm (channel DAPI); Image-it Green Dead: 488/515 nm (channel FITC); MitoTracker Red 579/599 nm (channel dsRED); Cell Mask Deer Red 650/655 (channel Cy5).

SAMPLE PREPARATION

Rat or human macrophages seeded onto black µclear 96-well plates are incubated with test material. After 24 h or 48 h of exposure, cells are stained and fixed following the cell health assay protocol (see SOP WP1/007 or WP1/008).

Plate layout:

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<td>B</td>
<td>CCM</td>
<td>Test 1: 0.01, 0.03, 0.1, 0.3, 1, 3.16, 10, 31.6 µM</td>
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<td>C</td>
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<td>Test 2: 0.01, 0.03, 0.1, 0.3, 1, 3.16, 10, 31.6 µM</td>
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<td>D</td>
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<td>Test 3: 0.01, 0.03, 0.1, 0.3, 1, 3.16, 10, 31.6 µM</td>
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<td>Triton -X</td>
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<td>E</td>
<td>CCM</td>
<td>Test 4: 0.01, 0.03, 0.1, 0.3, 1, 3.16, 10, 31.6 µM</td>
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<td>Test 5: 0.01, 0.03, 0.1, 0.3, 1, 3.16, 10, 31.6 µM</td>
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<td>Test 6: 0.01, 0.03, 0.1, 0.3, 1, 3.16, 10, 31.6 µM</td>
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**Fig. 1.** 96-well plate layout for *in vitro* drug challenge.

- Complete culture media (CCM)
- FCCP = positive control for mitochondrial activity
- Triton –X = positive control for membrane permeability
- Solubiliser: Dimethyl sulfoxide (DMSO) or phosphate buffered saline (PBS)
- Test 1-5 = Test material in serial dilution from lowest concentration (column 3) to highest concentration (column 10)

**IMAGE ACQUISITION:**

1. Switch on InCell Analyzer 6000.

2. Load a sample plate into instrument.

3. Within *Assay Development* mode, write an acquisition protocol using the *protocol designer* or alternatively open and edit the existing protocol: “UH Cell Health.xaqp”

4. In *Plate/Slide*, select the plate type from the drop down menu: µclear Greiner.

5. In *Objective Lens*, choose an objective (40x/0.45 NA).

6. In *Fields*, choose the number of *Fields to Acquire*: 12 fields, and *Field Placement*: random.

7. In *Channel Settings*, select the following:
   - Number of wavelengths: 4 (DAPI, FITC, dsRED and Cy5). Select the *Excitation* laser and *Emission* filter for each of the wavelengths.
   - Image size: Full size
   - Binning: 1x1
   - Link 3D parameters
   - Standard 2-D imaging mode
   - Start with exposure time of 0.100 s and Open Aperture
8. In **Focus Options**, select **Laser autofocus**, click on untreated well and optimize the focus and exposure time for each wavelength. Check the focus in a few treated wells and then go back to an untreated well before processing to the next step.

9. In **Acquisition options**, select **Horizontal serpentine**.

10. In **Plate view**, select wells for acquisition according to plate layout.

11. Run the acquisition protocol to acquire data from the plate.

12. Save the data on an external disk.