Kinetic and label-free, live content imaging assays for neurite outgrowth in primary, iPSC-derived and immortalised neurons

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Summary & Impact

• The study of neurite dynamics is fundamental to the investigation of neuropathological disorders, neuronal injury, regeneration, differentiation and embryonic development.
• Here we describe an in vitro fully kinetic neurite outgrowth assay miniaturised to 96 & 384-well microtiter plate formats based on analysis of time-lapse, phase-contrast images.
• Validation and pharmacology data from a range of cell types including human iPSC-derived neurons (iCell Neurons, CO2), primary neurons (rat cortex) and neuronal-like cell lines (Neuro-2A) are described. Neurite outgrowth (NOG) can be duplexed with cell count and cytotoxicity measurements using fluorescent probes.
• This approach affords a full temporal understanding of neurite outgrowth without the need for complex and expensive Ab-labeling methods (e.g. HCS).
• Miniaturisation of assays with iPSC-derived neurons (iCell, CO2) to 384-well format is a key step in maximising the value of these cellular reagents.

96 & 384-well NeuroTrack Assay – an Integrated solution

1. Seed selected neuronal cell type in the culture microplate (96 or 384-well) and allow adherence for 1h.
2. Treat cells with neuro-degenerate and/or neuroprotective agents.
3. Monitor in the IncuCyte ZOOM live-cell imaging system: capture and analyse phase and fluorescent images every 2-6h for 3 to 12 days.

Image processing & quantification

• Phase-contrast images and analysis masks show the kinetics of neurite outgrowth in rat cortical primary neurons.
• Time course: compare mean ± SEM neurite length (mm/mm²) and branch point (1/mm²) values for rat cortical, human (iCell Neurons and Neuro-2A cells) (n=6).

Validation: Cell body cluster normalisation

• Data represent mean ± SD values for rat cortical and human iCell Neurons (n=6 wells).
• Note how normalisation to cell body cluster allows for data comparison.

Nuclear-targeted GFP/RFP enables true cell counting

• Time course for NucLight Green and Red Neuro-2A cells differentiated using atRA (18 μM) showing the mean ± SEM (A, B) (n=9 wells).
• Phase and Fluorescence images obtained for NucLight Green and Red Neuro-2A cells (C, D) 5 days post-treatment.

Acknowledgement

We thank Cellular Dynamics International for technical support with iCell Neurons.

Kinetic multiplexed assays : NOG & neurotoxicity

A

Vehicle

0.1 nM [atRA] (M)

0.2 nM [atRA]

0.3 nM [atRA]

1 μM [atRA]

Neurite Length/CBC

Vehicle

[atRA] (M)

0.1 nM

0.2 nM

0.3 nM

1 μM

Log [Ro-31-8220]

AUC (NL/CBC)

AUC (Object count)

AUC (BP/CBC)

LOG (Neuro-2A Cell Death)

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