Quantitative, real-time live-cell analysis method and reagents for evaluation of cell health in neuronal cultures

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Introduction

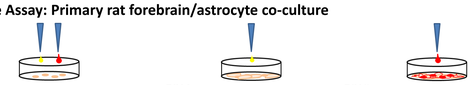
- Neuronal viability and neurite dynamics are key measures used to assess neurons in culture and in defining the neuronal phenotype. These measures are routinely used in neuroscience discovery to evaluate novel mechanisms/pathways involved in development or disease, neuroprotection as well as neurotoxic effects of novel test compounds.
- Many traditional approaches measuring cell viability and neurite dynamics rely on endpoint assays and labor intensive imaging techniques that require immunochemical staining.
- IncuCyte® Neuronal Assays feature automated, real-time multiplexed measurements of cell viability and neurite dynamics. In many cases, label-free readouts can be combined with non-perturbing live-cell reagents to simultaneously distinguish treatments that affect one or more aspects of neuronal cell health.

IncuCyte® ZOOM Imaging System

The IncuCyte® ZOOM Live-Cell Analysis System is a compact, automated optical instrument that resides inside your standard culture incubator and is used for long-term kinetic imaging in both HD-phase contrast and fluorescence. A key part of the IncuCyte® system is a robust software package that allows for automated data acquisition, image processing, and analysis.

Live Cell Analysis Workflow

Co-Culture Assay: Primary rat forebrain/astrocyte co-culture



DIV 0:
Plate primary rat forebrain neurons 15K or 45K neurons/well. Add NeuroLight after 4 hrs for Fluorescent NeuroTrack experiment

DIV 1:
Plate primary rat astrocytes 15K or 20K astrocytes/well

DIV 9:
Add Glutamate CRC ± MK-801; Cytotox or Annexin Reagent
Scan every 6 hrs – out to DIV 13

Monoculture Assay: Neuro-2a Cell Line

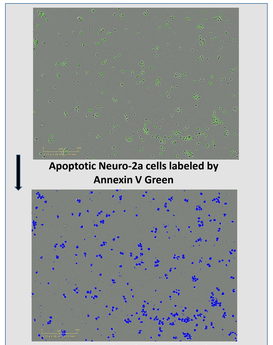
DIV 0:
Plate Neuro2A cells 4K cells/well in 10% serum

DIV 0 + 4 hrs:
Feed with 20μM retinoic acid, 2% serum (differentiation); U0126 CRC and Annexin Reagent
Scan every 6 hrs – out to DIV 3

DIV - Day in Vitro

Automated Image Analysis

- IncuCyte® ZOOM Live-Cell Analysis System enables observation and quantification of cell behavior over time by automatically capturing the full kinetic response in the biological model.
- Application-specific automated image processing tools based on user-defined parameters.
- Imaged based approach for visual inspection of cell morphology and metric verification.
- Increase productivity and throughput - capacity to run experiments in six, 96- or 384-well plates at one time with full solution protocols and analysis modules.
- Maintain experimental conditions with label-free analysis options and non-perturbing reagent formulations.

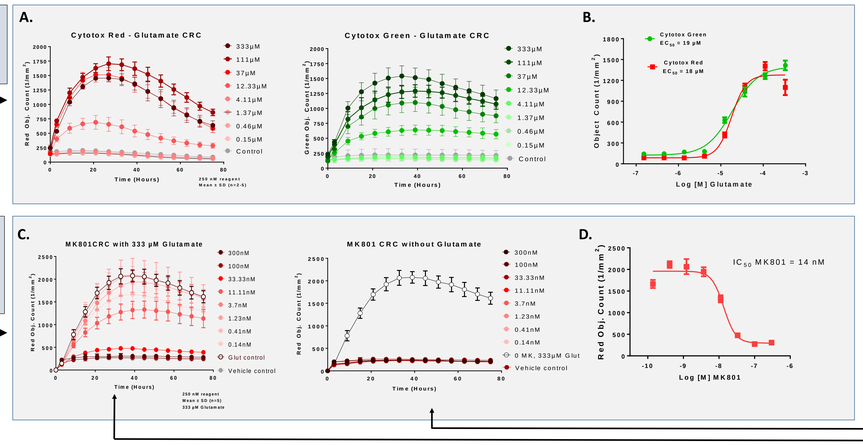


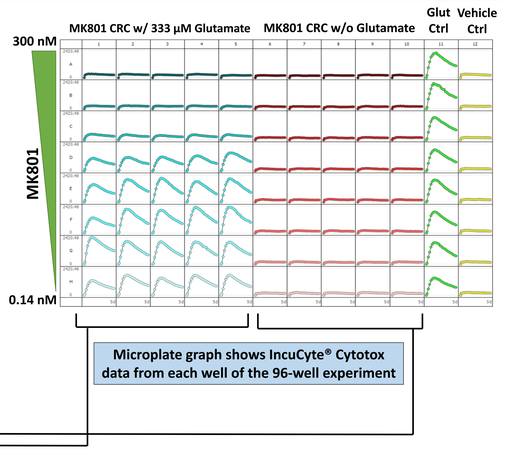
Evaluation of Neuronal Cell Health Following Glutamate-Induced Excitotoxicity using IncuCyte® Cytotox Red and Green Reagents – Primary Neuron Co-Culture

- **A.** Glutamate produced a time- and concentration-dependent increase in Green and Red Object Count (time expressed as post addition on DIV 9)
- **B.** Single time-point analysis done at peak response (24 hrs) - Glutamate EC_{50} 18 μM (Cytotox Green) and 19 μM (Cytotox Red)
- **C.** Glutamate-induced excitotoxicity reduced by co-application of the NMDA receptor antagonist MK801
- **D.** MK-801 IC_{50} = 14 nM for reducing Glutamate-induced cell death

Kinetically measure Glutamate toxicity →

Kinetically measure NMDA receptor block →

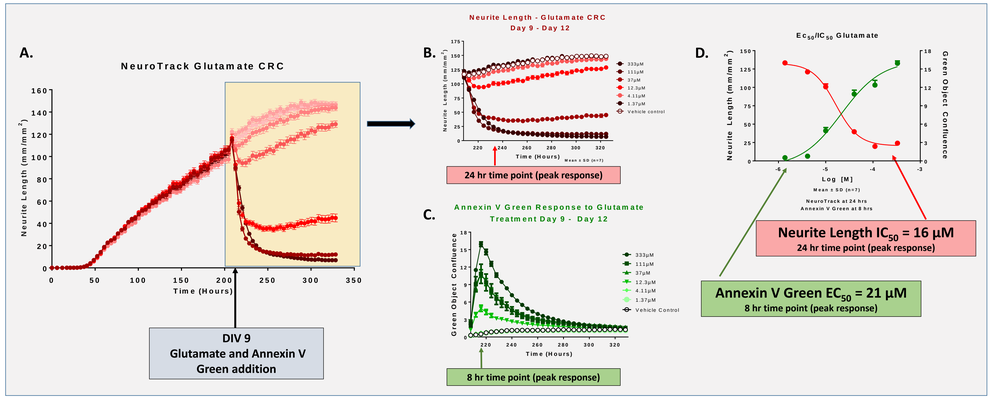


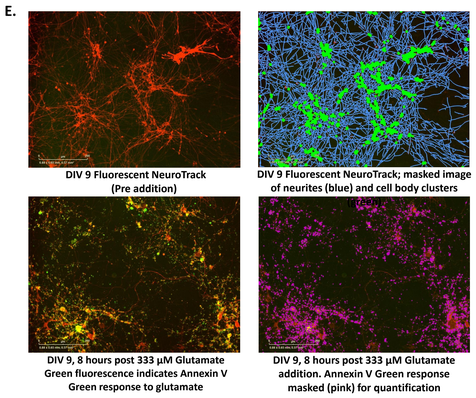


Microplate graph shows IncuCyte® Cytotox data from each well of the 96-well experiment

Multiplexing Neuronal Cell Health and Neurite Dynamics with IncuCyte® Annexin V Green Reagent – Primary Neuron Co-Culture

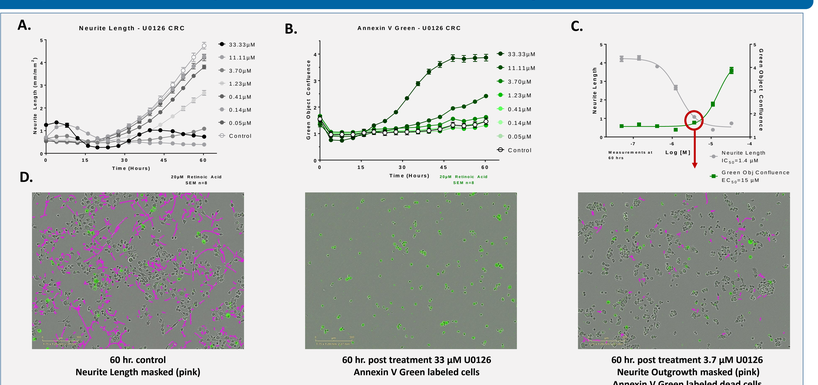
- **A.** Fluorescent Neurite Length tracks neuronal network development - DIV 9 (208 hrs) chosen for glutamate addition to evaluate network disruption
- **A. and B.** Addition of glutamate produced a concentration-dependent reduction in Neurite Length
- **C.** Multiplexed within the same experiment, glutamate produced a concentration-dependent increase in Annexin V Green signal, indicating activation of apoptotic cell death pathways
- **D.** Single time-point analysis done at peak response to glutamate – Neurite Length (24 hrs) - Glutamate IC_{50} = 16 μM; Apoptotic cell death (8 hrs) EC_{50} = 21 μM (Annexin V Green)
- **E.** Sample images from a **single** well at DIV 9 before and after glutamate addition showing masking/quantification of neurite length and Annexin response





Differential Effect of U0126 (MEK1/MEK2 Inhibitor) on Cell Health and Neurite Dynamics – Neuro-2a Cells

- **A**. U0126 produced a concentration-dependent reduction in Neurite Length (Phase NeuroTrack)
- **B.** Higher concentrations of U0126 produced an Annexin V Green response, indicating compromised cell health
- **C.** Single time-point analysis done at 60 hrs post U0126 treatment – Neurite Length - IC_{50} = 1.4 μM; Apoptotic cell death EC_{50} = 15 μM (Annexin V Green)
- **D.** Sample images at 60 hrs showing phase contrast masking of neurites (left), Annexin V Green labeled cells from high conc of U0126 (center) and mid conc of U0126 (3.7 μM) showing reduced neurite length with minimal increase in Annexin Green labeled cells (right)



Summary

- Continuous, long-term live cell analysis with IncuCyte® Cytotox and Annexin V reagents was used to quantify glutamate-induced excitotoxic cell death in primary rat forebrain/astrocyte coculture.
- IncuCyte® Annexin V Green was multiplexed with Fluorescent NeuroTrack Neurite Dynamic measurements to evaluate glutamate-induced apoptotic cell death and reduction in neurite length in a single experiment.
- U0126, a dual MEK1/MEK2 inhibitor, reduced neurite length at 10x lower concentrations than those that produced apoptotic cell death (Annexin V Green) in differentiated Neuro-2a cells.
- These data demonstrate the utility of the IncuCyte® ZOOM as a unified platform for evaluation of neuronal cell health and neurite dynamics with Cytotox and Annexin V reagents along with the NeuroTrack analysis software in a kinetic live cell analysis approach.