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Janet L. Anderl¹, S¹Bioprocess Science Division, High Content Analysis Research and Development, Millipore Inc

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Analysis of Neurotoxicity

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Isolation and Functional Analysis of Mitochondria from Cultured Cells and Mouse TissueThomas Lamp¹, Jo A. Crum¹, Taylor A. Davis¹, Carol Milligan^{2,3,4}, Victoria Del Gaizo Moore¹¹Chemistry Department, Elon University, ²Department of Neurobiology and Anatomy, Wake Forest School of Medicine, ³Neuroscience Graduate Program, Wake Forest School of Medicine, ⁴ALS Center Translational Science Unit, Wake Forest School of Medicine

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PROTOCOL

Cell Preparation:

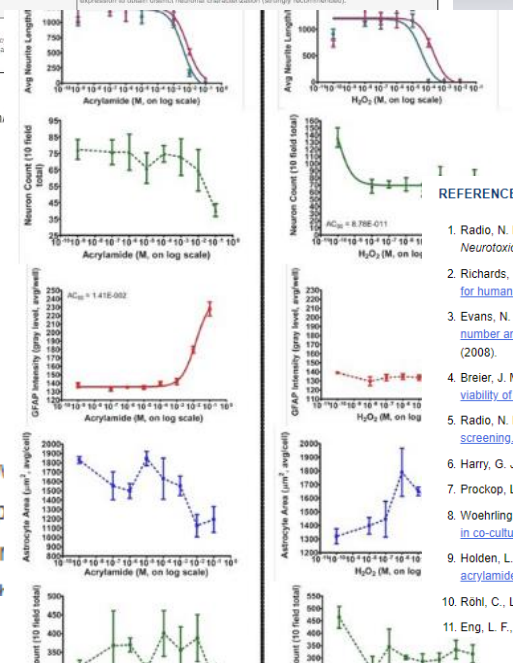
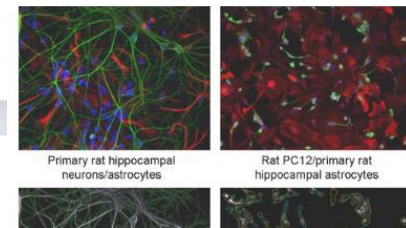
1. Prior to cell seeding for assay, culture neurons/astrocytes in growth media until ~70-80% confluent (unless cell seeding directly from thaw or isolation).
2. Detach cells from culture flasks/plates via method appropriate for cell type of interest. If necessary, coat assay plate wells with poly-D-lysine or extracellular matrix protein to enhance cell adhesion. Co-cultures of astrocytes and neurons may be seeded simultaneously or sequentially. For sequential seedings, plating of astrocytes first as a "basal" layer is recommended, followed by neuronal plating and differentiation, if necessary. Adjust cell density as appropriate for cell type, subsequent culture time, parameters of interest, etc. Depending on culture age, cell source and seeding density, primary cultures may vary greatly in rate of proliferation, GFAP expression or neurite outgrowth - it is important to characterize and optimize your cell system to provide the most biological relevance for your experimental model, as well as to provide for effective image segmentation and analysis using i described in Step 3 below), allow period, incubate cells in growth m PC12 cells), as appropriate. Conti for cell type.
3. Cell treatments (control compound of treatment of interest. Acrylamid for duplicate 12-point dose respor compounds are provided at 250X peroxide, assuming maximum tre; dilution of the 250X compound in be serially diluted directly in sterik already present in each well, for a treatment at 37°C prior to fixation.

i. *Exp.* (27), e1173,

Cell Parameter	Detection	Segmentation/Measurement	Rationale
Cell Number, Nuclear Characteristics	Hoechst/HCS Nuclear Stain	Nuclear region (488 nm emission channel). Count number of nuclei. DNA content (nuclear intensity) or nuclear area analyses are also possible.	Use cell number, nuclear characteristics to determine cell loss, toxicity phenotypes, etc. Can "filter" nuclei for those associated with β -tubulin or GFAP expression to obtain separate neuronal and astrocytic cell counts/characterizations (strongly recommended).
β -III-Tubulin Expression, Neurite Outgrowth	HCS Secondary Antibody, FITC-conjugated	Cytoplasmic region (535 nm emission channel). FITC signal may be used to distinguish neuronal cell bodies from neurites (e.g., via minimum/average cell body area), minimum/maximum neurite lengths and widths). Determine parameters such as total neurite length, neurite count/total, etc.	Neurite outgrowth measurements may be modulated during neuronal differentiation or as a result of chemical injury/disease states, etc. Can "filter" cell bodies for those associated with β -tubulin expression to obtain distinct neuronal characterization (strongly recommended).
GFAP Expression, Astrocyte Area	HCS Secondary Antibody, Cy3-conjugated	Cytoplasmic region (630 nm emission channel). Cy3 signal may be used to define astrocyte segmentation. Determine parameters such as average cytoplasmic signal intensity, cell a	

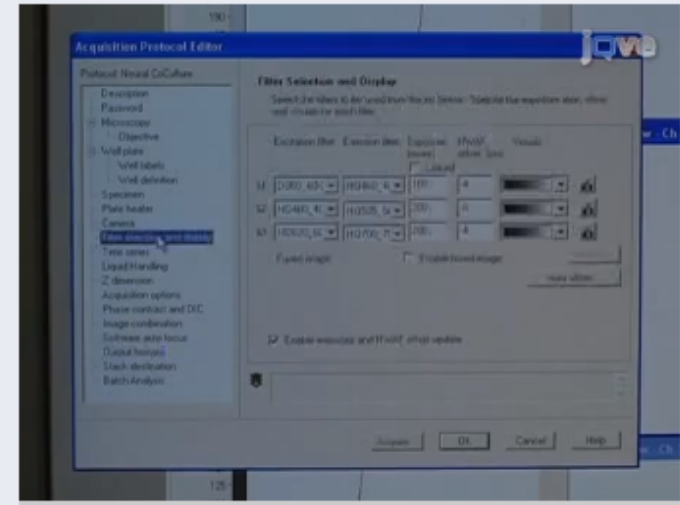
Table 1. Image Acquisition and Analysis Guidelines – HCS222 β -III-Tubulin.

Representative Results:



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ABSTRACT

High Content Analysis (HCA) assays combine cells and detection reagents with automated imaging and poi measurement of multiple cellular phenotypes within a single assay. In this study, we utilized HCA to develop assessment represents an important part of drug safety evaluation, as well as being a significant focus of er neurotoxicity is also a well-accepted in vitro marker of the development of neurodegenerative diseases such

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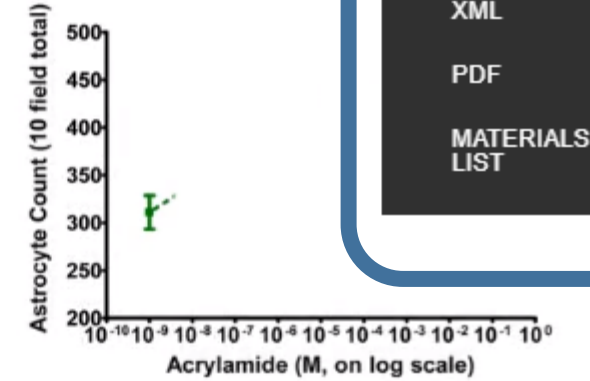
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A Neuronal and Astrocyte Co-Culture Assay for High Content Analysis of Neurotoxicity

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Abstract

High Content Analysis (HCA) assays combine cells and detection reagents with automated imaging and powerful image analysis algorithms, allowing measurement of multiple cellular phenotypes within a single assay. In this study, we utilized HCA to develop a novel assay for neurotoxicity. Neurotoxicity assessment represents an important part of drug safety evaluation, as well as being a significant focus of environmental protection efforts. Additionally, neurotoxicity is also a well-accepted *in vitro* marker of the development of neurodegenerative diseases such as Alzheimer's and Parkinson's diseases. Recently, the application of HCA to neuronal screening has been reported. By labeling neuronal cells with β III-tubulin, HCA assays can provide high-throughput, non-subjective, quantitative measurements of parameters such as neuronal number, neurite count and neurite length, all of which can indicate neurotoxic effects. However, the role of astrocytes remains unexplored in these models. Astrocytes have an integral role in the maintenance of central nervous system (CNS) homeostasis, and are associated with both neuroprotection and neurodegradation when they are activated in response to toxic substances or disease states. GFAP is an intermediate filament protein expressed predominantly in the astrocytes of the CNS. Astrocytic activation (gliosis) leads to the upregulation of GFAP, commonly accompanied by astrocyte proliferation and hypertrophy. This process of reactive gliosis has been proposed as an early marker of damage to the nervous system. The traditional method for GFAP quantitation is by immunoassay. This approach is limited by an inability to provide information on cellular localization, morphology and cell number. We determined that HCA could be used to overcome these limitations and to simultaneously measure multiple features associated with gliosis - changes in GFAP expression, astrocyte hypertrophy, and astrocyte proliferation - within a single assay. In co-culture studies,

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