Neurite Outgrowth Assay for High Content Screening

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Abstract

Major drug discovery efforts are focused on identification of compounds affecting neurite outgrowth, as neurite formation/neuronal regeneration holds therapeutic promise for a number of neuronal diseases, including stroke, and diabetic neuropathy. High Content Screening (HCS) for measurement of neurite outgrowth stimulation. Treat with activators/inhibitors of neurite outgrowth outgrowth. In addition, we show that the assay reagents are stable for 24 hr at room temperature, enabling high-quality screening applications. The assay provides the opportunity to perform non-subjective, quantitative neurite outgrowth assays.

Introduction

Introduction to in vitro drug discovery research are focused on the identification of compounds that affect neurite outgrowth. The development of High-Content Screening (HCS) technology represents a major step forward in improving the drug discovery and phenotypic imaging and analysis of neurite outgrowth and neuronal cell morphology. The assay is immunofluorescence-based, and uses a primary antibody which specifically labels neurites and neuronal cell bodies from a wide variety of mammalian species, including human, mouse and rat. Sufficient reagents are provided are to screen 96 wellplates. The assay can be used effectively for screening both inducers and inhibitors of neurite outgrowth.

Millipore and Drug Discovery

Millipore provides a broad range of products and services to enable accelerated drug discovery.

Typical HCS Workflow

Sample Preparation

Automated Image Acquisition

Data Analysis and Interpretation

Automated Image Analysis

Results

Plate cells on microplates suitable for HCS imaging

Treat with activators/inhibitors of neurite outgrowth

Fix cells using HCS Fixation Solution (30 min)

Rinse cells with Neurite Outgrowth HCS Immunofluorescence Buffer

Perform immunofluorescent procedures using Neurite Outgrowth HCS Immunoreagents (2.5 hr)

Perform imaging and analysis using HCS reader e.g. GE Cell Scout, Cellomics ArrayScan, Konica Open

Methods

1. PC12 cells were plated on ECM protein-coated clear-bottom plates suitable for HCS imaging, at a concentration of 2,000-4,000 cells/well.

2. After 24 hr, growth media was replaced with fresh serum-free media containing neurite growth factors (NGF).

3. Cells were cultured for 6 days under different differentiation conditions, replacing media at 3-4 day intervals. Neurite outgrowth began to develop after 4 days in differentiation media. 6 days in differentiation media for all groups. Samples were fixed in 4% paraformaldehyde immediately before immunofluorescence.

4. Primary antibody which specifically labels neurites and neuronal cell bodies from a wide variety of mammalian species, including human, mouse and rat. Sufficient reagents are provided are to screen 96 well plates. The assay can be used effectively for screening both inducers and inhibitors of neurite outgrowth.

Related Products from Millipore

GP208: Nerve Growth Factor (NGF) - beta

NS220: Neurite Outgrowth Assay H1 (3 µm)

NS221: Neurite Outgrowth Assay H1 (1 µm)

HCS100: CellCube® Cytotoxicity Assay: HeLa cells, for HCS

Summary

1. Millipore’s Neurite Outgrowth Assay for High Content Screening enables effective morphological screening of neurite outgrowth.

2. Assay offers the opportunity for higher-throughput, non-subjective, quantitative neurite outgrowth assays.

3. Primary antibody is highly specific for neurites and neuronal cell bodies, and reacts with a wide variety of mammalian species, including human, mouse and rat.

4. Reagents are stable for at least 24 hr at room temperature, facilitating large scale screening applications.

5. Assay can be used to screen for neurite outgrowth, as neurite formation/neuronal regeneration holds therapeutic promise for a number of neuronal diseases, including stroke, and diabetic neuropathy.

6. Assay can be used to screen for compounds which inhibit neurite outgrowth or neuronal cell bodies from a wide variety of mammalian species, including human, mouse and rat. Sufficient reagents are provided are to screen 96 well plates. The assay can be used effectively for screening both inducers and inhibitors of neurite outgrowth.

7. Assay can be used to screen for promoters of neurite outgrowth.

8. Assay can be used to screen for compounds which induce neurite outgrowth.

9. Assay can be used to screen for compounds which inhibit neurite outgrowth.

10. Assay provides the opportunity to perform non-subjective, quantitative neurite outgrowth assays.

11. Assay provides the opportunity to perform non-subjective, quantitative neurite outgrowth assays, and enables high-throughput, non-subjective, quantitative neurite outgrowth assays. The assay is immunofluorescence-based, and uses a primary antibody which specifically labels neurites and neuronal cell bodies from a wide variety of mammalian species, including human, mouse and rat. Sufficient reagents are provided are to screen 96 well plates. The assay can be used effectively for screening both inducers and inhibitors of neurite outgrowth.

12. Assay provides the opportunity to perform non-subjective, quantitative neurite outgrowth assays, and enables high-throughput, non-subjective, quantitative neurite outgrowth assays. The assay is immunofluorescence-based, and uses a primary antibody which specifically labels neurites and neuronal cell bodies from a wide variety of mammalian species, including human, mouse and rat. Sufficient reagents are provided are to screen 96 well plates. The assay can be used effectively for screening both inducers and inhibitors of neurite outgrowth.

13. Assay provides the opportunity to perform non-subjective, quantitative neurite outgrowth assays, and enables high-throughput, non-subjective, quantitative neurite outgrowth assays. The assay is immunofluorescence-based, and uses a primary antibody which specifically labels neurites and neuronal cell bodies from a wide variety of mammalian species, including human, mouse and rat. Sufficient reagents are provided are to screen 96 well plates. The assay can be used effectively for screening both inducers and inhibitors of neurite outgrowth.

14. Assay provides the opportunity to perform non-subjective, quantitative neurite outgrowth assays, and enables high-throughput, non-subjective, quantitative neurite outgrowth assays. The assay is immunofluorescence-based, and uses a primary antibody which specifically labels neurites and neuronal cell bodies from a wide variety of mammalian species, including human, mouse and rat. Sufficient reagents are provided are to screen 96 well plates. The assay can be used effectively for screening both inducers and inhibitors of neurite outgrowth.