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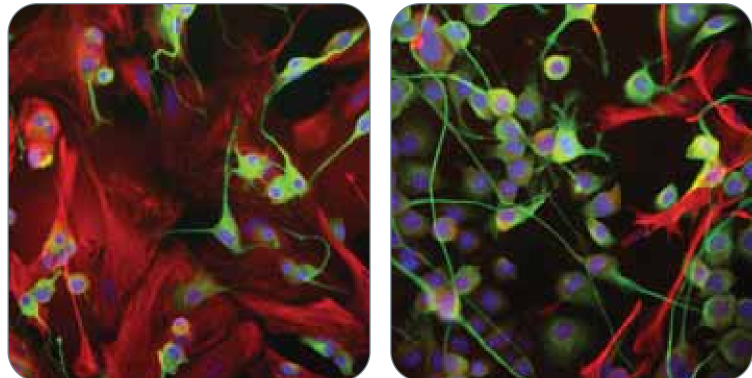
Neurite Outgrowth Assay

Quantify Neurite Outgrowth Using High Content Screening

Millipore's new Neurite Outgrowth Assay is specifically designed for High Content imaging and analysis of neurite outgrowth and neuronal cell morphology. The assay is immunofluorescence-based, and uses a high quality primary antibody that specifically labels neurites and neuronal cell bodies from a wide variety of mammalian species, including human, mouse and rat. The reagents and protocols contained within the kit provide a complete solution for specifically labeling neurites and neuronal cell bodies for High Content imaging.

Advantages

- **High sensitivity** generating images with a high signal-to-background ratio, facilitating subsequent High Content Analysis
- **Neuronal specific reagents** identify neuronal cell bodies and neurites in heterogeneous cell populations
- **Assay reagents are stable** at room temperature for at least 24 hours simplifying large scales screening efforts
- **High-quality, validated, target-specific detection reagents** for profiling neurite outgrowth and neuronal morphology in a wide variety of mammalian cell types
- **Ideal for screening** both inducers and inhibitors of neurite outgrowth and neurotoxins



Specificity of Neurite Outgrowth Primary Antibody

Rat PC12 pheochromocytoma (A) or mouse N1E-115 neuroblastoma (B) cells were co-cultured with rat hippocampal astrocytes on 96-well clear-bottom plates. Samples were fixed in Millipore's HCS Fixation Solution before immunofluorescent staining for neuronal cells (FITC (green) secondary antibody, under HCS200 assay conditions) and astrocytes (glial-cell specific GFAP with rhodamine (red) secondary antibody). Nuclei are counterstained with Hoechst HCS Nuclear Stain (blue) under kit conditions (20X objective).

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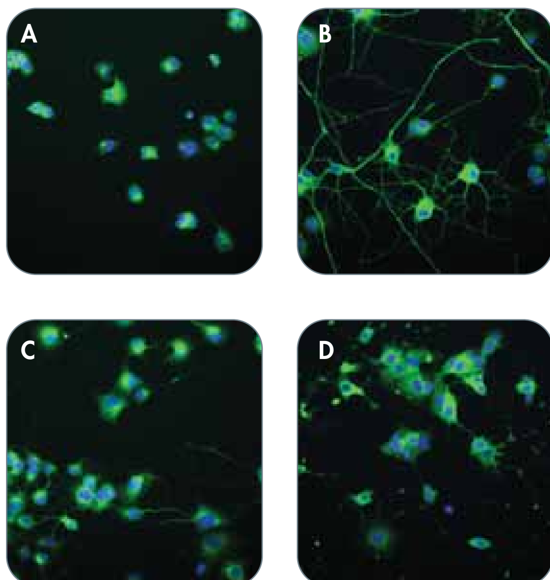


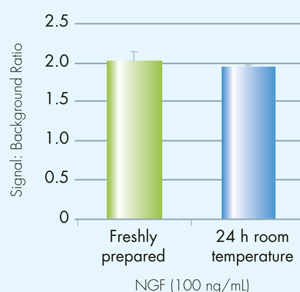
Immunofluorescence of treated and untreated PC12 cells

Merged images of staining with Hoechst HCS Nuclear Stain (blue) and Neurite Outgrowth Primary/Secondary Antibodies (green).

A. Unstimulated PC12 cells. Cells were cultured in low serum differentiation media containing no NGF for 6 days, replacing media every 3 days.

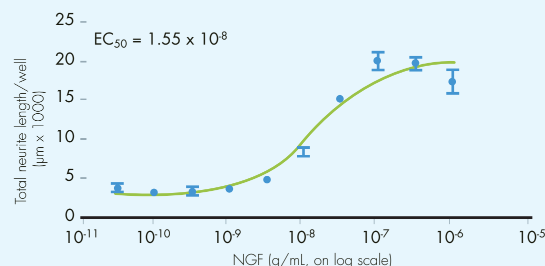
B. NGF-stimulated PC12 cells. Cells were cultured in low serum media containing 100 ng/mL NGF for 6 days, replacing media/NGF every 3 days.

C & D. NGF-stimulated PC12 cells treated with neurite outgrowth inhibitors. Cells were cultured in low serum media containing 100 ng/mL NGF for 6 days, replacing media/NGF every 3 days. Cells received treatment with either the protein kinase inhibitor K252a (0.3 µM), for the final 3 days of culture (C), or the microtubule depolymerization agent nocodazole (11 µM), for the final 4 hours of culture (D). Cell handling, fixation and immunostaining were performed according to HCS200 assay protocols.



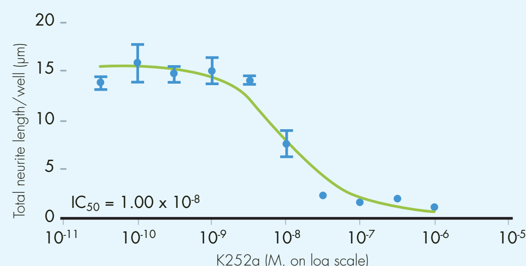
Neurite Outgrowth HCS reagent stability

PC12 cells were seeded under conditions promoting neurite outgrowth. Samples were fixed and stained under kit conditions, using either fresh buffers and antibody/Hoechst solutions, or buffers and antibody/Hoechst solutions that had been allowed to sit on the bench (protected from light) at room temperature for 24 hours prior to staining. Cells were imaged on the GE IN Cell Analyzer 1000 (3.3) and analyzed for cell (FITC) signal:background ratios using the GE IN Cell Analyzer 1000 Workstation (3.4) Multi Target Analysis algorithm. No statistically significant difference was observed between freshly prepared and 24 hour samples.



Dose response of NGF-induced neurite outgrowth stimulation

PC12 cells were cultured in low serum differentiation media containing serial dilutions of NGF (max. concentration = 1000 ng/mL) for 6 days, replacing media/NGF every 3 days, then fixed and stained. Cells were imaged on the GE IN Cell Analyzer 1000 (3.3) at 20X (5 fields/well) and analyzed using the GE IN Cell Analyzer 1000 Workstation (3.4) Neurite Outgrowth algorithm. (Mean ± SEM; n = 4)



Dose response of K252a- and nocodazole-induced neurite outgrowth inhibition

PC12 cells were cultured in low serum differentiation media, containing 100 ng/mL NGF for 6 days, replacing media/NGF every 3 days. Cells received treatment with either serial dilutions of the protein kinase inhibitor K252a, for the final 3 days of culture (max. concentration = 1000 nM), or the microtubule depolymerization agent nocodazole, for the final 4 hours of culture (max. concentration = 35 µM). Cells were imaged on the GE IN Cell Analyzer 1000 (3.3) at 10X (10 fields/well) and analyzed using the GE IN Cell Analyzer 1000 Workstation (3.4) Neurite Outgrowth algorithm. (Mean ± SEM, n = 4)

Neurite Outgrowth Assays

Description	Quantity	Cat. No.
Neurite Outgrowth Assay	5 x 96 well plates	HCS200
CellCiphr™ Cytotoxicity Assay, HepG2 cells, for HCS *(available July 2007)*	16 cmpd	HCS100
Nerve Growth Factor NGFβ	20 µg	GF028
Neurite Outgrowth Assay Kit (3 µM)	1 Kit	NS220
Neurite Outgrowth Assay Kit (1 µM)	1 Kit	NS225

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