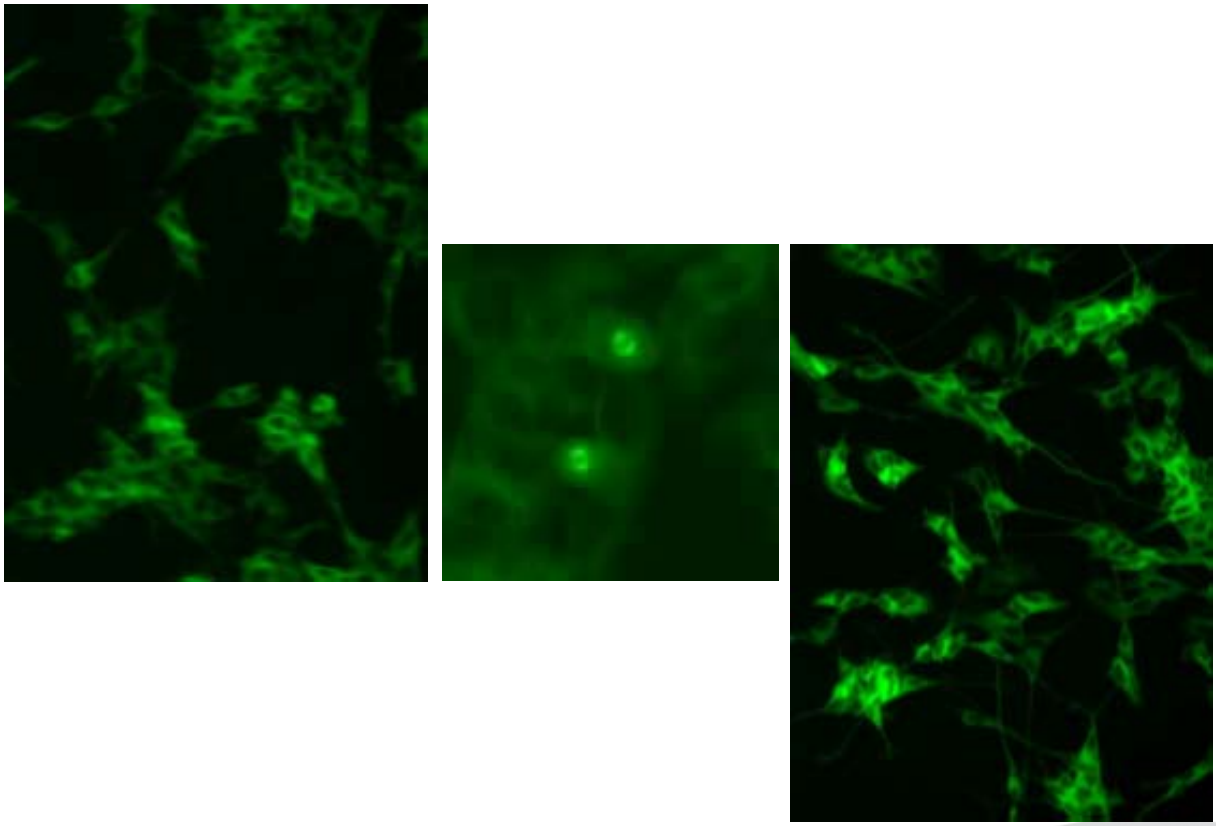
REF: P30709

INNOPROT ASSAYS FOR HIGH-CONTENT SCREENING

NEURODEGENERATIVE DISEASES IN VITRO MODELS

- NEURITE OUTGROWTH & MITOSIS ASSAY CELL LINE -



Product name: Neurite Outgrowth & Mitosis Assay Cell Line

Ec_{50} Paclitaxel (Mitosis Assay): 22.5 nM

Z´ (Mitosis Assay): 0.60+/- 0.01

V.A.T. Nº: ESB95481909

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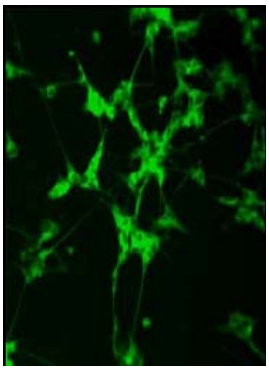
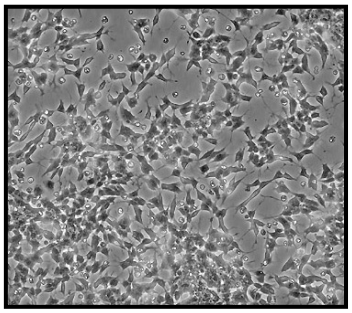
NEURITE OUTGROWTH & MITOSIS ASSAY CELL LINE

TagGFP2-TUBULIN SH-SY5Y CELL LINE



Product Name:	TagGFP2-Tubulin SH-SY5Y Cell line
Catalog Number:	P30709
Protein tagged:	Alpha(1b)-Tubulin
Fluorescent Protein:	TagGFP2
Format:	3 x 10^6 cells in Cryopreserved vials
Storage:	Liquid Nitrogen

A novel green fluorescent SH-SY5Y cell line has been developed through stable transfection with tubulin tagged Evrogen TagGFP2. This cell line expresses green fluorescent tubulin marking the cell cytoskeleton.



TagGFP2-Tubulin SH-SY5Y Cell line is stably transfected clonal cell line that is ready to use in cell-based assay applications. This stably transfected clonal cell line provides consistent levels of expression, which helps simplify the interpretation of results. This cell line is intended to be used as "in vitro" model for neuronal differentiation assays and monitoring the cellular mitosis level through microtubule structural changes.

Applications

NEURITE OUTGROWTH: The growth of neurites in human neurons is a critical event in neuronal development, formation and remodelling of synapses, response to injury, and regeneration. The discovery of new compounds that can positively affect neuritogenesis would be very important for developing new therapeutics against both neurodegenerative diseases and injury.

MITOSIS ASSAY: Mitosis is a process that occurs in eukaryotic cells and proceeds to cell division, consisting of the equitable sharing of genetic material. During mitosis, microtubules are nucleated to form the mitotic spindle by polymerizing soluble tubulin in order to attach chromosomes and then separate them into daughter cells

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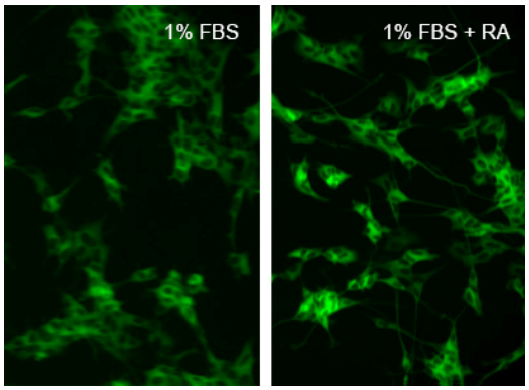
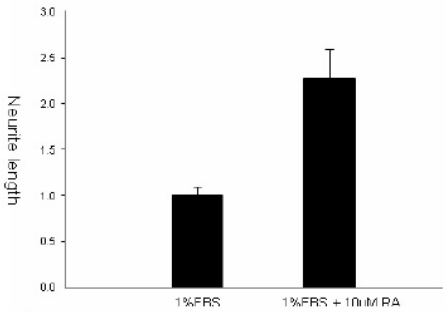
Assay Details I

NEURITE OUTGROWTH:

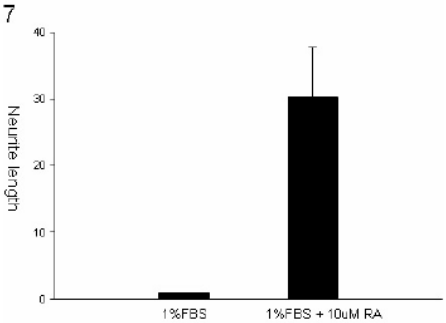
SH-SY5Y stably expressing human tubulin tagged in the C-terminus with TagGFP were stimulated with 10 µM of retinoic acid during 7 days. After 3 and 7 days, cells were analyzed with the Becton Dickinson Pathway 855 High Content Bioimager using the Neurite Outgowth application of the Attovision Software. When cells were treated with the retinoic acid, we observed an increase in the length of the developed neurites. The activity was calculated as an increment of neurite length.



Day 3



Day 7



MITOSIS ASSAY:

This cell line has been also designed to assay compounds for their ability to affect the cell cycle analyzing the microtubule organization. To validate this cell line, TagGFP2-tubulin/SH-SY5Y cells were treated with Paclitaxel. Paclitaxel stabilizes microtubules and prohibits further polymerization and depolymerization. The suppression of microtubule dynamics may prevent chromosomes from moving from the spindle poles to the metaphase plate slowing or preventing progression from metaphase to anaphase and cells enter a state of mitotic arrest.

The assay was developed and optimized using the BD Pathway HCS Reader and Attovision Compartimentalization Software.

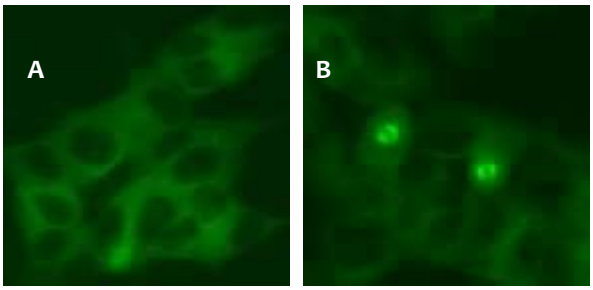


Fig.1. TagGFP2-tubulin without (A) and with 0.1 µM (B) Paclitaxel

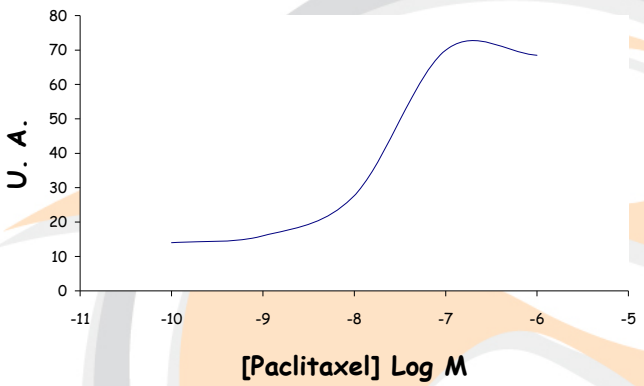


Fig.2. Mitotic spindle quantification after 1 hour of treatment. In this curve, the Ec50 for Paclitaxel was 22.5 nM. This assay was validated with an average of Z'= 0.60+/- 0.01 for High Content Screening.

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