cyprotex AN EVOTEC COMPANY

*In vitro* **Toxicology**

# Neurite Outgrowth

## Background Information

'Neurite outgrowth is a requisite for an accurate functional network of neurons during development. It is also crucial for neuronal plasticity, as well as neuronal regeneration.'

[1]Salto R *et al.*, (2015) *PLoS One* **10(8)**; e0135614

- The neurite outgrowth assay uses human iPSC-derived neurons (other cell types available on request).
- Cyprotex's neurite outgrowth assay uses high content screening technology to monitor neurite outgrowth.
- Neurons are plated on laminin-coated 384-well plates 1 hr prior to treatment. After treatment with test articles and control compounds, neurons are maintained in a humidified environment at 37°C with 5% $CO_2$ for 72 hr (optimal). At the end of the treatment period, cell are fixed, permeabilised and stained for evaluation of neurite outgrowth and cell health.
- This assay provides a viable *in vitro* system for assessing compounds that interfere or promote normal neurite outgrowth in neurons, providing a platform for preclinical drug safety, drug discovery and disease modelling.

## Protocol

**Cell Type**
Human iPSC-derived neurons (other cell types available upon request)

**Analysis Platform**
ArrayScan VTi or CellInsight CX7 (Thermo Scientific)

**Test Article Concentrations**
10 point dose-reponse curves in triplicate (dependent on customer requirements)

**Quality Controls**

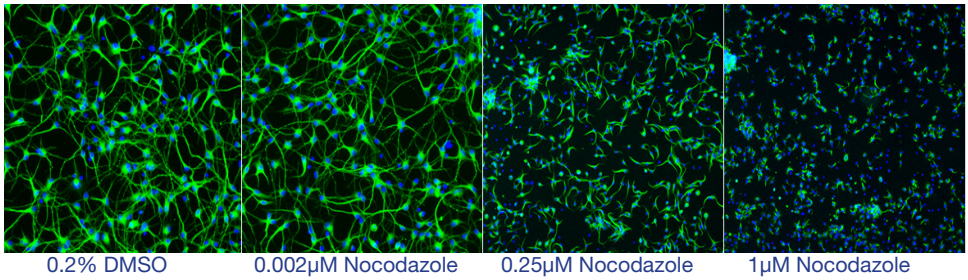
*Negative Contols:*
0.2% DMSO (vehicle)
Chlorpromzine

*Positive Control*
Nocodazole

**Data Delivery**
This assay has been optimised to assess cell health and neurite outgrowth utilising a neuronal profiling bioapplication. Valid cell count, mean neurite average length and neurite total length per neuron are reported.

## Figure 1

High content images of human iPSC-derived neurons after 72 hr treatment with nocodazole over a range of concentrations.

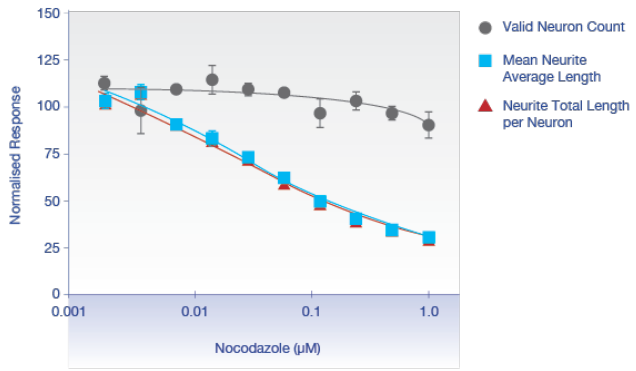


0.2% DMSO | 0.002µM Nocodazole | 0.25µM Nocodazole | 1µM Nocodazole

Images show a decrease in neurite outgrowth in a dose dependent manner while valid cell count is not significantly affected.

## Figure 2

Evaluation of cell health and neurite outgrowth for positive control nocodazole tested in human iPSC-derived neurons.

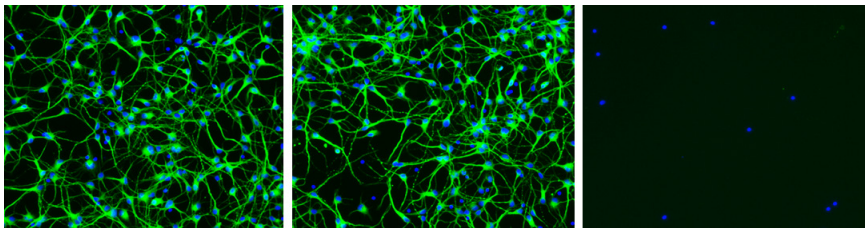



| | $EC_{50}$ (µM) |
|---|---|
| Valid neuron count | > 1 |
| Mean neurite average length | 0.04933 |
| Neurite total length per neuron | 0.03191 |

Positive control nocodazole causes a significant decrease in mean neurite average length and neurite total length per neuron over the range of concentrations tested. Nocodazole did not have significant effects on cell viability at these concentrations.

## Figure 3

High content images of human iPSC-derived neurons after 72 hr treatment with vehicle control (0.2% DMSO) and 0.2 µM and 100µM chlorpromazine.

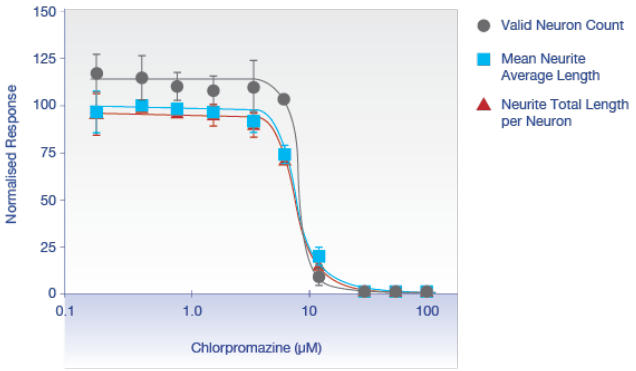


0.2% DMSO | 0.2µM Chlorpromazine | 100µM Chlorpromazine

At 100µM, chlorpromazine causes cell death. At the lowest concentration of 0.2µM, cell loss and neurite outgrowth are unaffected. Chlorpromazine has no effect on neurite outgrowth independent of cell death.

## Figure 4

Evaluation of cell health and neurite outgrowth for negative control, chlorpromazine, tested in human iPSC-derived neurons.




| | $EC_{50}$ (µM) |
|---|---|
| Valid neuron count | 8.645 |
| Mean neurite average length | 8.950 |
| Neurite total length per neuron | 8.383 |

Negative control chlorpromazine causes a significant decrease in mean neurite average length, neurite total length per neuron and valid neuron count over the range of concentrations tested. There is a direct correlation between cell loss and neurite length.


**References**

[1] Salto R *et al.* (2015) ß-Hydroxy-ß-methylbutyrate (HMB) promotes neurite outgrowth in neuro2a cells. *PLoS One* **10(8)**; e0135614