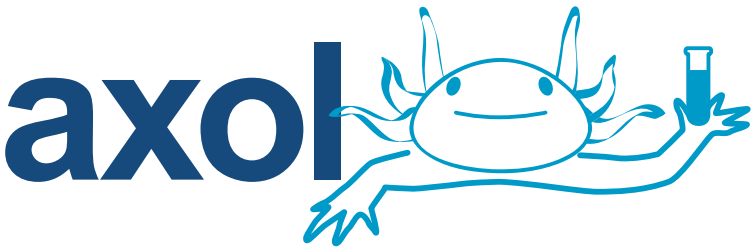
Cell Player™ IncuCyte ZOOM – NeuroTrack assay using Axol hNPCs

Application Protocol

Version 2.1



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Study of Neurite Dynamics using NeuroTrack Assay

The study of neurite dynamics is elemental to the investigation of neuropathological disorders, neuronal injury and regeneration, embryonic development, and neuronal differentiation. Measurements of neurite dynamics are routinely used as a screening assay in neurotherapeutic drug discovery, and changes in neurite length and branching can predict neurotoxicity and neuroprotective effects induced by a compound.

The predominant method for studying neurite dynamics has been to concatenate measurements of neurite length and branch points taken at single points in time. This approach involves growing neuronal cultures, exposing them to a treatment condition, and halting the experiment with fixation and immunostaining steps. Afterwards, image acquisition and analysis can be performed. Although this method has been an important tool in neurobiology, the process is labor-intensive and produces only a single timepoint of data, which may leave dynamic effects of the treatment unrevealed.

To resolve these issues, the NeuroTrack™ assay was developed. NeuroTrack software, coupled with the IncuCyte ZOOM™ live-content imaging platform, quantifies living cells' neurite dynamics without requiring a fluorescent label. The IncuCyte ZOOM takes non-perturbing phase-contrast images of neuronal cultures under physiological conditions over an extended period of time. NeuroTrack software produces data on neurite dynamics by analysing these images.

Importantly, users can obtain data from their culture for a complete time course, potentiating thorough understanding of the kinetic effects of the treatment of interest. NeuroTrack is compatible with a variety of neuronal model systems; among others, the software is capable of quantifying the very fine neurites of primary and iPSC derived cells, and the robust processes of differentiated Neuro-2a and PC-12 cells

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cellplayer™ ASSAYS & REAGENTS

Preparation of Axol hNPCs for NeuroTrack Assay

Axol human neural progenitor cells (hNPCs) are derived from integration-free, induced pluripotent stem (iPS) cells under fully defined neural induction conditions. hNPCs can be thawed, plated and differentiated on the first day of the NeuroTrack assay; alternatively, they can be expanded ahead of differentiation, or they can be dissociated and re-plated post-differentiation to suit your experimental requirements.

Product Name:	Catalog no.	Vendor
Axol hNPCs	**ax0013 - ax0016**	Axol Bioscience
Axol AD hNPCs	**ax0111 - ax0115**	Axol Bioscience
Axol Neural Maintenance Medium Kit (Includes ax0031 & ax0031b)	**ax0031 (a&b)**	Axol Bioscience
Axol Sure Bond	**ax0041**	Axol Bioscience
Axol Neural Advance™	**ax0042**	Axol Bioscience
Axol Neural Unlock™	**ax0044**	Axol Bioscience
Axol Sure Boost™	**ax0045**	Axol Bioscience
Axol Neural Enhance™	**ax0046**	Axol Bioscience
96-well assay plate. Techno Plastic Product Plates are recommended.	93096	TPP
WFI-grade water	9999-0002-A	Mediatech
PBS (without calcium and magnesium)	14190169	Life Tech

For thawing, passaging and differentiation instructions, please refer to our Culture and Differentiation of Axol hNPCs instruction manual at:

http://www.axolbio.com/instruction-manuals.html

Cell seeding to a 96-well plate (NeuroTrack Assay)

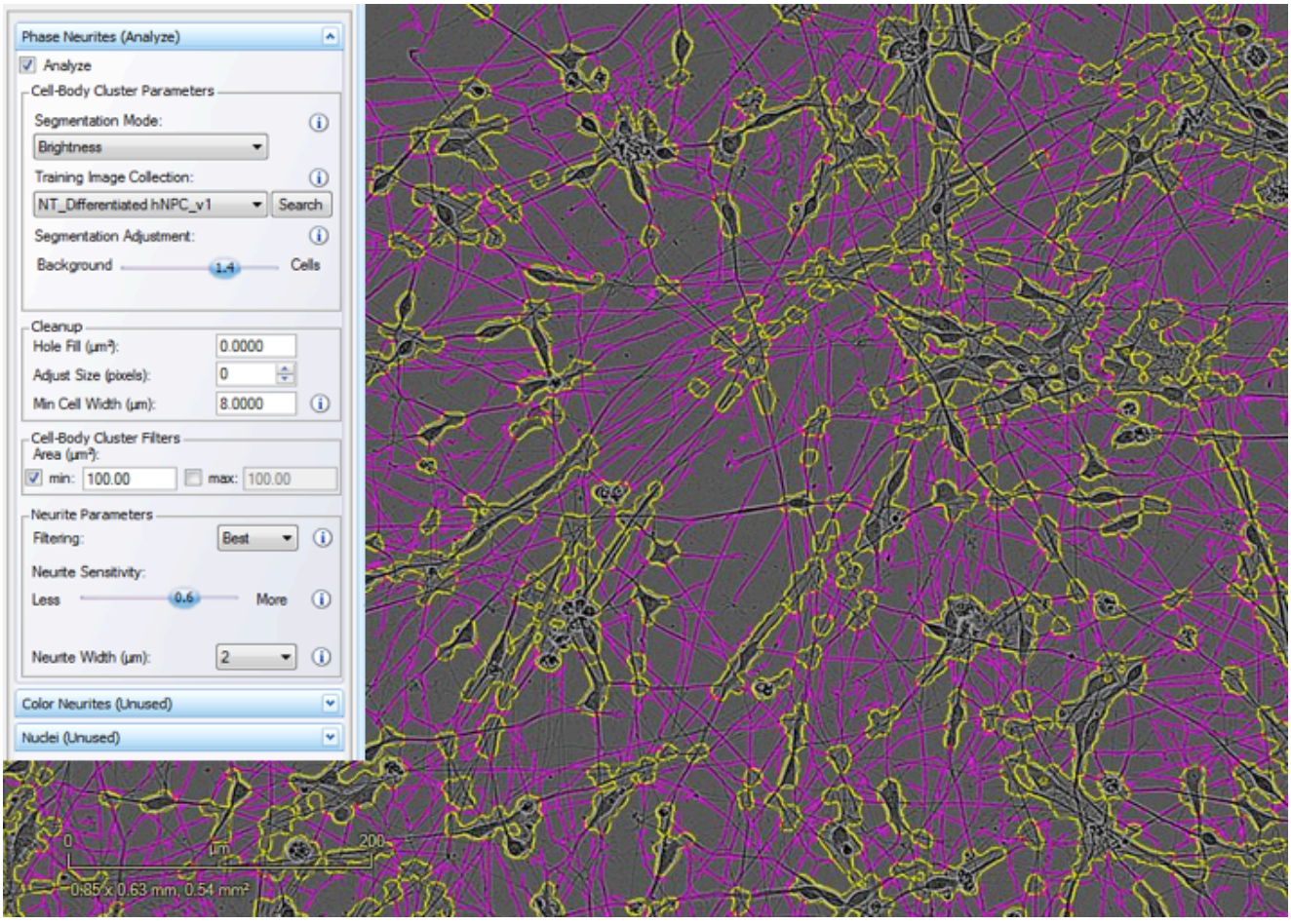
1. Thaw the **Axol Sure Bond™** coating solution **overnight at 4°C**.
2. Dilute the **Axol Sure Bond™** stock solution **(50X)** WFI-grade water to make **working solution** **e.g. 120 µL in 6 mL**.
3. Coat the surface each well with **100 µL Axol Sure Bond™** working solution.
4. Incubate the culture vessel **overnight at 37°C**.
5. Remove and rinse wells once with PBS immediately before the cells need plating.
6. For a 96-well assay, adjust density to 100K cells/ml in 1:1000 **Axol Sure Boost™** supplemented media and plate **100 µl per well** (10K cells/well) in the coated 96-well TPP plate.
7. Leave at RT for **30 min** to minimise edge effects.
8. Place plate in IncuCyte ZOOM and monitor neurite dynamics for the duration of the experiment.
9. 24h post seeding remove the neural enhance supplemented media and replace with pre-warmed 1:1000 **Axol Neural Advance™** supplemented media.
10. 2 days post-differentiation replace 50% of the media (i.e 50 µl) with 2x FAC **Axol Neural Advance™** (1:500) for an extra 2-3 days.
11. After this time perform a 100 % media replacement with Complete Axol Neural Maintenance Medium. At this point treatments may be added if necessary.
12. Replace 50% of media every 2-3 days.

Data Analysis

Export the raw data to Excel (Neurite length (mm/mm^2, Branch points (1/mm^2) and normalization to cell body cluster) and re-arrange accordingly to treatments.

Use GraphPad Prism 5.0 to plot changes in the analysed parameter as function of time (hours). Obtain the area under the curve from this time-course data.

Use Excel to plot the log of molar concentration of test compound against the effect of each compound on the area under the curve. Perform a nonlinear regression, using the 4 parameter logistic model (fitting model 204), to yield estimates of compound pIC$_{50}$ values, Hill coefficient and minimum and maximal values (XLfit Version 5.1.0.0.).



Technical Support

- Online Resources

  Please visit our website at www.axolbio.com for additional product information and *Technical Resources*, including instruction manuals, application protocols, video guides, wall charts and webinars.

- Contact Us

  For more information or technical assistance, call +44 (0) 1223 497 119, or email support@axolbio.com. US Toll Free Tel: 1-800-678-2965 (1-800-678-AXOL), US Toll Free Fax: 1-800-861-2965 (1-800-861-AXOL).

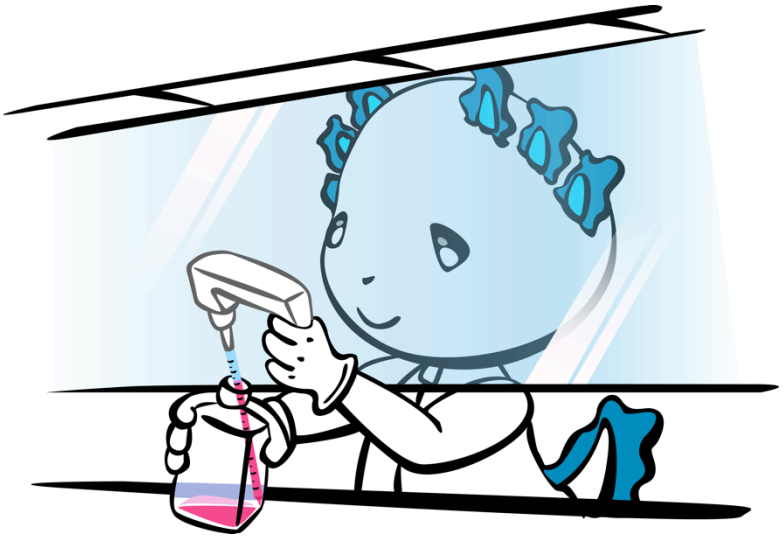
- Certificate of Analysis

  The Certificate of Analysis provides detailed quality control information for each product. Certificates of Analysis are available on our website.

  Go to www.axolbio.com/certificate-of-analysis-lookup and search for the Certificate of Analysis with product lot number, which is printed on the cryovial label.

  For any questions related to CellPlayer™ IncuCyte ZOOM- NeuroTrack assay please visit Essen Bioscience for support

  http://www.essenbioscience.com/support.html



Don’t forget to rate, review and register your Axol product at www.axolbio.com