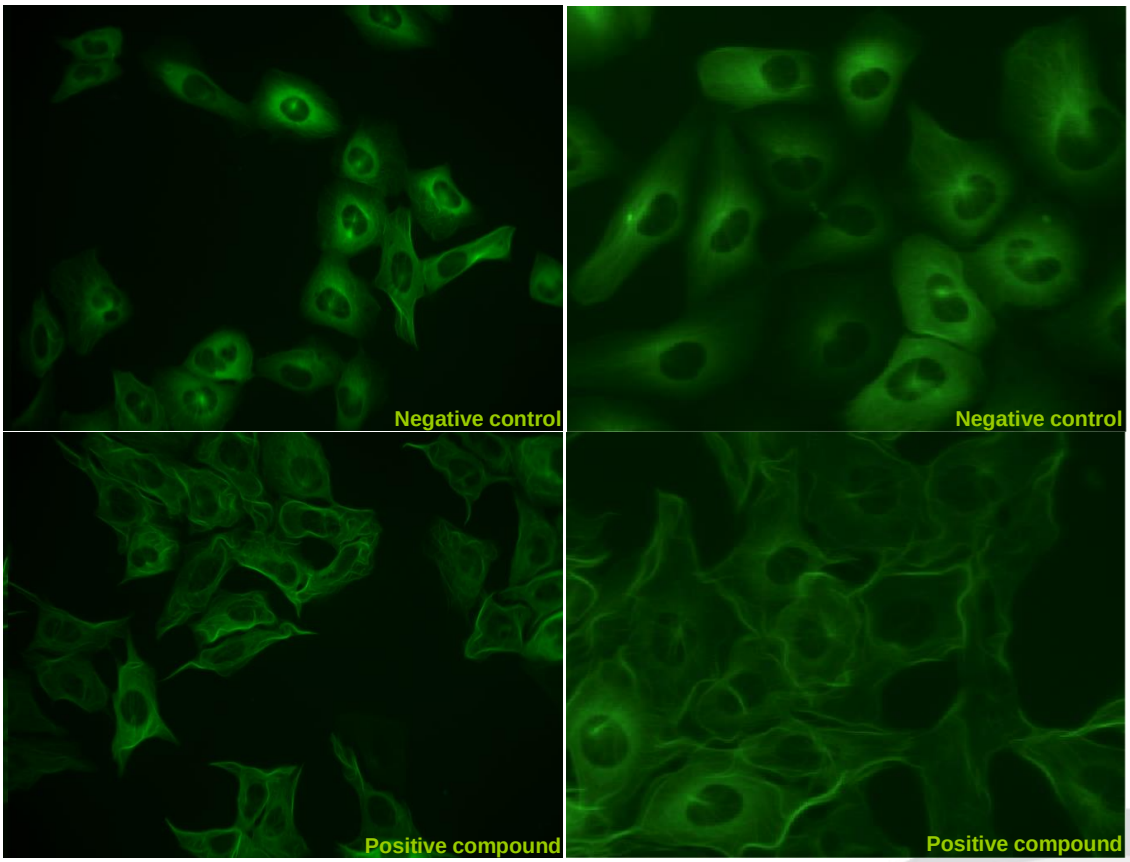
REF: P30705

INNOPROT ASSAYS FOR HIGH CONTENT SCREENING

ALZHEIMER'S DISEASE IN VITRO MODELS

- TAU PHOSPHORYLATION ASSAY CELL LINE -



Product name: TauTM-tGFP / U2OS cell line

IC_{50} LiCl: 11.75 mM

Z´: 0.80+/- 0.02

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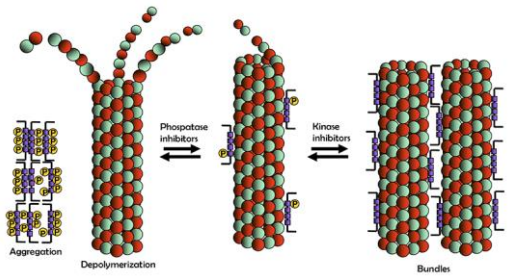
ALZHEIMER'S DISEASE *IN VITRO* MODELS

- TAU PHOSPHORYLATION ASSAY -

Cell Line Name:	Tau0N4R-TM-tGFP U2OS Stable Cell line
Pathway:	Tau De/Phosphorylation – Microtubule De/Binding
Assessment:	Tau & Microtubule Bundle Formation Assessment
HCS Application:	Fluorescent Bundle Quantification
Material provided:	***P30705***: Stable Cell Line (2 vial of cells)
	P30705-DA: Division-Arrested Cells (2 million cells)

Background

The microtubule associated protein Tau is the main component of the neurofribillary tangles (NFT), aberrant structures that appear in the brain of Alzheimer´s disease patients and other tauopathies. Tau protein binds to and stabilizes microtubules (MTs) but in pathological states, hyperphosphorylation and truncation of the protein leads to its aggregation. A novel recombinant cell line has been developed for the screening of kinase modulators that affect the behaviour or location of Tau protein.



Cell Line Characteristics

Multiple Tau gene mutations are pathogenic for hereditary FTDP-17 disease. These mutations have similar effects to hyperphosphorylation in Tau in AD and result in NFT formation. U2OS stably expressing triple mutant (TM) human 0N4R Tau-tGFP allows to perform an assay to evaluate endogenous Tau kinases and phosphatases in living cells. Binding of Tau to MTs and consecuent bundle-formation is phosphorylation dependent: hyperphosporylation of Tau leads to its dissociation from microtubules and aggregation into tangles of paired helical filaments (PHF). On the contrary, kinase inhibitors promote Tau binding to microtubules and the formation of microtubule bundles. This bundle increase is detected and quantified by fluorescence using automated image analys.

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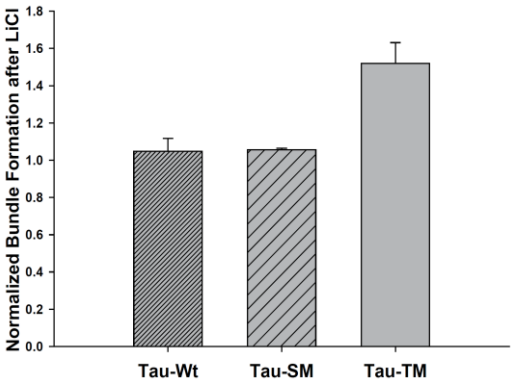
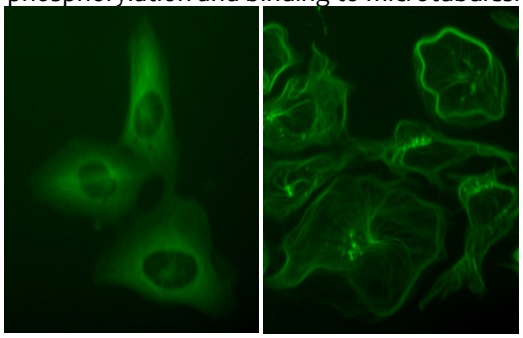
Assay Characterization

This assay was also performed with human Tau wild-type and single mutant Tau. Depending on the mutation, MT-bundle amount varies. These Tau mutations have similar effects to hyperphosphorylation: the triple mutant has less affinity for microtubules and consequently forms less bundles. Wt and SM shows more bundling cells than TM at rest, so their capacity to form bundles after GSK3-β kinase inhibitor LiCl addition is limited.



Assay Validation

U2OS stably expressing the human triple mutant Tau-tGFP cells were treated with log dilution series (n=3) of the LiCl during 2 hours to evaluate their effects on Tau phosphorylation and binding to microtubules.

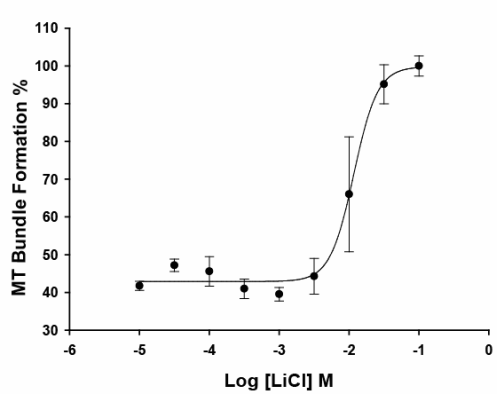


Negative control | LiCl treated cells 2 h

After that, the nucleus was stained with DAPI and cells Tau and MT bundles were detected by fluorescence using image analysis algorithms. % Activity was calculated relative to positive (100 mM).

Determination of IC50 value

Dose-response curve for the GSK3-β inhibitor LiCl.



IC50 value for LICl was determined by treating of U2OS TauTM-tGFP model cells with inhibitor concentrations from 10uM to 100mM during 2 hours. Followed this incubation, the intracellular bundle formation is quantified with a BD Pathway 855 High-Content Bioimager and Attovision software. Error bars represent the standard deviation among 3 replicate wells. Ic50 for LiCl is 11.75 mM and z' for this experiment was 0,80 +/-0,02.

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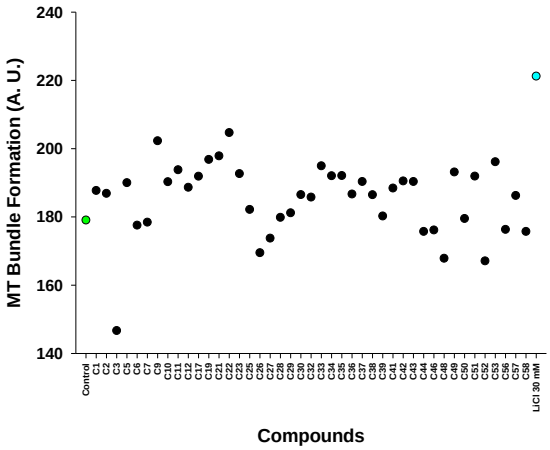
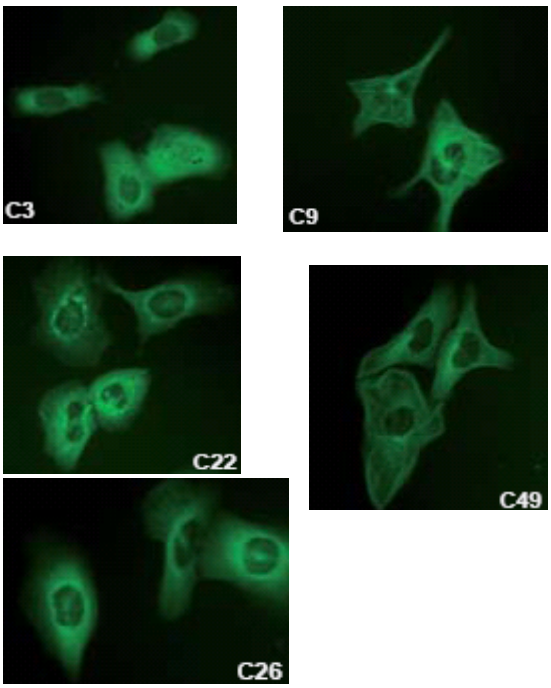
Screening Campaign

Tau and microtubule bundle formation screening of representative and less toxic compounds from a library was performed. Compound were screened at 10 uM of concentration. The positive control (LiCl 30 mM) is represented in cyan. The negative control is represented in green color. Some of the compounds hav e effect on Tau distribution: C3 and C26 shows no binding to MTs and in some cells Tau appears in nucleus; C9 and C49 shows more MT bundles; C22





This Alzheimer's Disease in vitro model based in U2OS can be used in drug discovery for Tau kinase and phosphatase inhibitors and modulators.

This model have been adapted to HCS analyses based in image algorithms to test processing effects .

This model permits evaluate a lybrary of compounds, candidates to inhibitors, in living cells studying the vesicles retention.

This model allows to analyse in the space and time the compound effect in a multiparametric manner.

This model provide a strategy to evaluate drugs againts kinase and phosphatase activity without the necessity to be permeable.

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