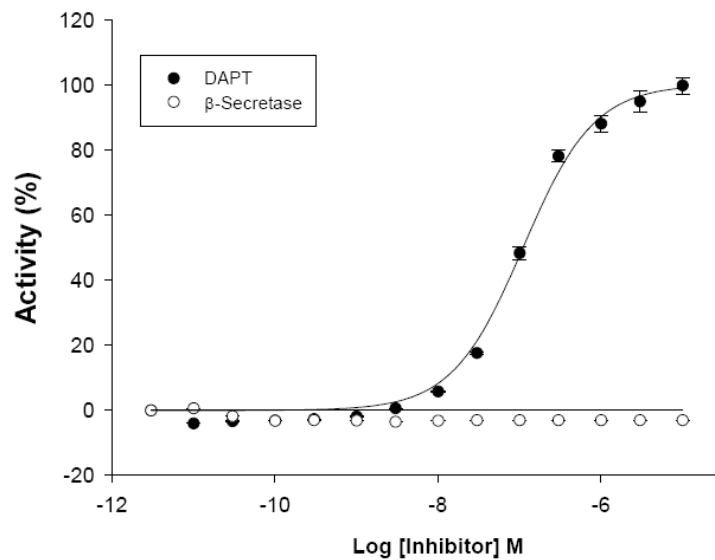
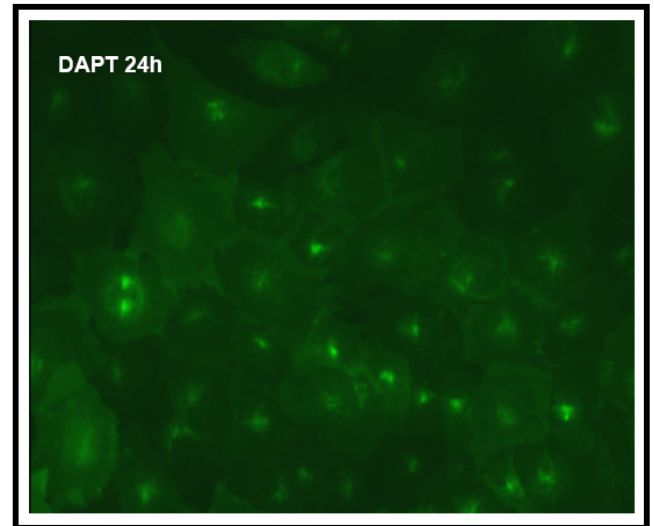
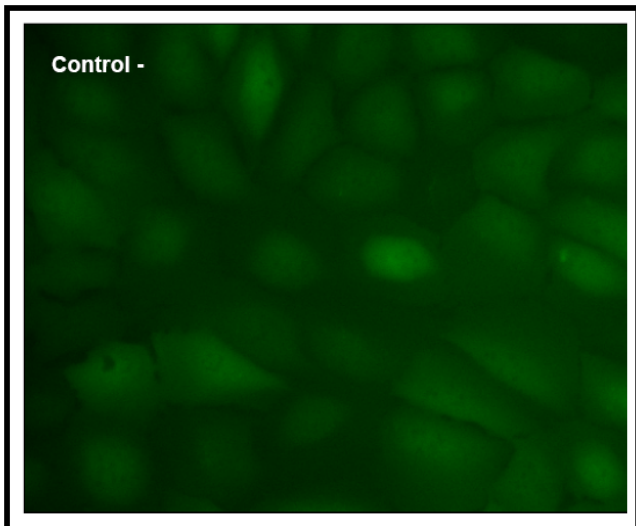


INNOPROT ASSAYS FOR HIGH CONTENT SCREENING

ALZHEIMER'S DISEASE IN VITRO MODELS

- GAMMA-SECRETASE ACTIVITY ASSAY CELL LINE -



Product name: γ -secretase Assay Cell Line

Cell line construction: APP-C99-tGFP / U2OS cell line

IC₅₀ DAPT: 1.10x10⁻⁷ M

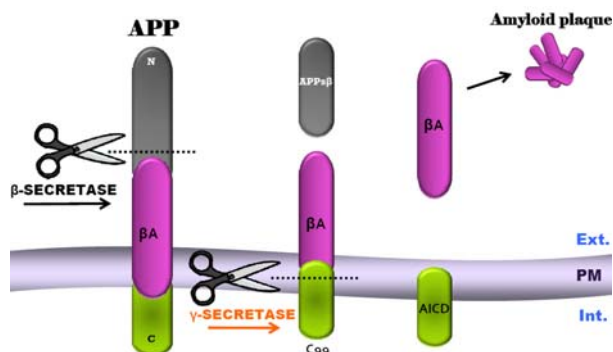
ALZHEIMER'S DISEASE *IN VITRO* MODELS - γ -SECRETASE ACTIVITY ASSAY -

Cell Line Name:	APP-C99-tGFP U2OS Stable Cell line
Pathway:	APP Processing – A β Secretion
Assessment:	γ -Secretase Activity Assessment
HCS Application:	Fluorescent APP-C99 Aggregates formation
Material provided:	P30702: Stable Cell Line (2 vial of cells) P30702-DA: Division Arrested cells (2 million cells per vial)

This cell line has been produced with the technology developed within FP7 PASCA EU project, and is 100% certified truly monoclonal.

Background

Alzheimer disease (AD) is characterized by brain depositions of the beta amyloid (β A). The β A is the amyloid precursor protein (APP) digestion product, which is released from the cell after β -secretase and γ -secretase proteolysis. A novel recombinant cell line expressing a fluorescent construct (APP-C99) containing the γ -secretase cleaving site has been developed for screening inhibitors for γ -secretase activity via APP processing pathway.



Cell Line Characteristics

APP-C99 U2Os cells allow to perform assays to evaluate the endogenous γ -secretase proteolytic process in living cells. This cell line has been validated using different inhibitory compounds for γ -secretase (positive controls) and β -secretase activity (negative controls).

High content analysis of γ -secretase activity has been designed to be performed using an epifluorescent imaging system to acquire and analyze images and to quantify the fluorescent vesicles into the cytoplasm.

The results obtained during the assay validation indicate that the pharmacological inhibition of γ -secretase implicated in AD is a valid strategy for drug screening and these models are appropriate to monitor the disease process *in vivo* in bioimaging systems.

Assay Validation

U2OS cells stably expressing APP-C99-tGFP construct were treated with DAPT, a γ -secretase inhibitor and indirectly of Notch. DAPT has been shown to dose-dependently decrease amyloid- β levels via inhibition of γ -secretase in both plasma and cerebral spinal fluid.

These cells have been also treated with beta-Secretase Inhibitor IV, an inhibitor of β -secretase to be used as a negative control.

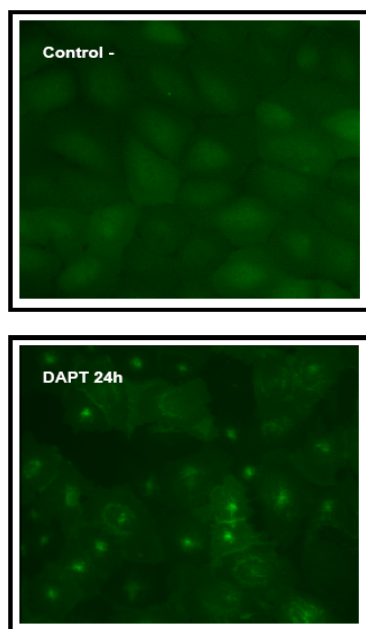


Fig1. Aggregates formation with DAPT inhibition of γ -secretase.

Determination of IC₅₀ value

Dose-response curve for both β -secretase and γ -secretase inhibitors.

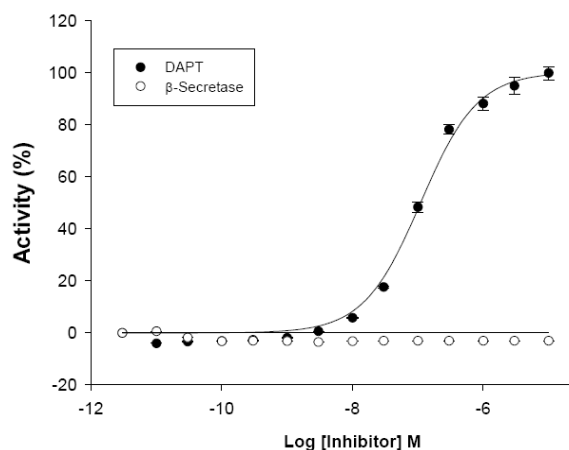


Fig2. IC₅₀ value for DAPT was determined by treating U2OS APP-C99-tGFP cells with inhibitor concentrations from 100pM to 100 μ M during 24 hours. Aggregates formation was quantified with a BD Pathway 855 High-Content Bioimager and Attovision software. IC₅₀ for DAPT is 1.10×10^{-7} M and z' for this experiment was $0,79 \pm 0,02$.

Use Restriction

This product contains a proprietary nucleic acid coding for a proprietary fluorescent protein intended to be used for research purposes only. No rights are conveyed to modify or clone the gene encoding fluorescent protein contained in this product, or to use the gene or protein other than for non-commercial research. For information on commercial licensing, contact Licensing Department, Evrogen JSC, email: license@evrogen.com