INNOPROT ASSAYS FOR HIGH CONTENT SCREENING
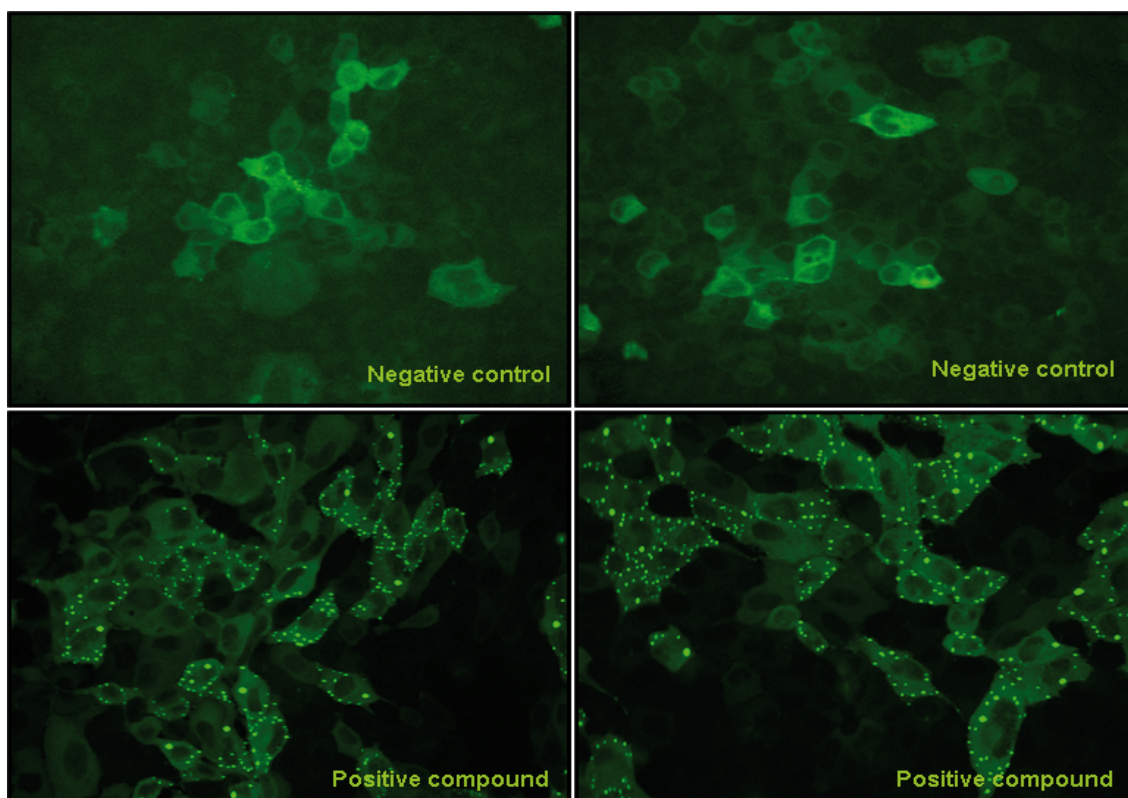
ALZHEIMER'S DISEASE IN VITRO MODELS

- APP PROCESSING ASSAY CELL LINE -

Product name: APP-tGFP / MDCK cell line

ic_{50} **LP226A1:** 17,80 uM

Z´: 0.90+/- 0.01

**ALZHEIMER'S DISEASE *IN VITRO* MODELS**

- APP PROCESSING ASSAY -

Cell Line Name:	tGFP-APP MDCK Stable Cell line
Pathway:	APP Processing – Aβ Secretion
Assessment:	γ & β Secretase Activity Assessment
HCS Application:	Fluorescent APP Vesicles Quantification
Material provided:	P30701: Stable Cell Line (2 vial of cells)
	P30701-DA: Division Arrested cells (2 million cells/vial)

Background

Alzheimer disease (AD) is characterized by brain depositions of the beta amyloid (βA). The βA 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 has been developed for screening inhibitors for both secretases activity via APP processing pathsway.

βA secretion pathway - activated secretases

APP vesicle retention pathway - inhibited secretases
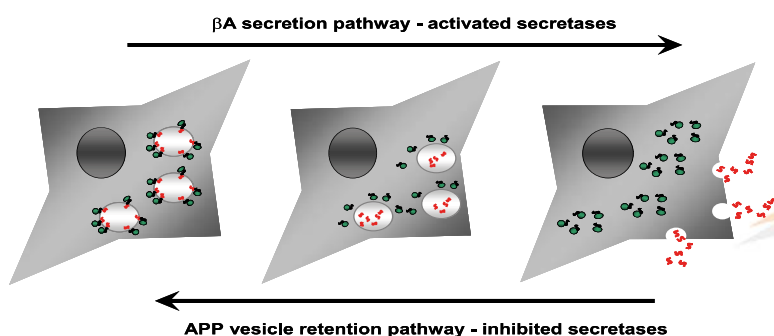
Cell Line Characteristics

tGFP-APP MDCK cells allow to perform assays to evaluate the endogenous secretase proteolityc process in living cells. This cell line has been validated using different inhibitory compounds and a siRNA for BACE to block the γ-secretase & β-secretase activities to decrease their processing. Each batch of Division-Arrested Cells has been highly validated showing the same response to the reference compounds than stable cell line.

High content analysis of γ and β 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 citoplasm. The results obtained during the assay validation indicate that the pharmacological inhibition of secretases 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

MDCK stably expressing human APP-tGFP cells were treated with 6 log dilution series (n=4) of inhibitors during 72 hours. Inhibitors used were DAPT and L-685,458 as γ-secretase inhibitors, β-secretase inhibitor IV and Batimastat as α-secretase inhitor.
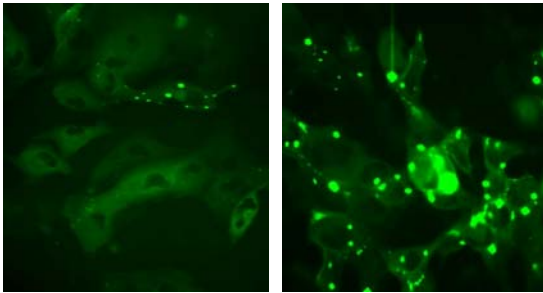

| Negative control | β-secretase inhibitor IV treated cells – 72 h |

After that, the nucleus was stained with DAPI and cells with retained APP spots were detected by fluorescence using image analysis algorithms. % Activity was calculated relative to positive (10 μM). This assay was also performed with a 96 hours tratment.
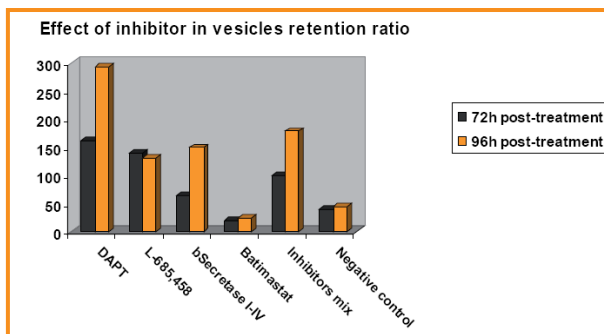

The results indicate that the detecting dynamyc range is dependent on the inhibitor biophysics and biochemical characteristics and the treatment time. This retention assay was validated with an average of Z´= 0.71+/- 0.01 for High Content Screening with a 72 hours treatment.
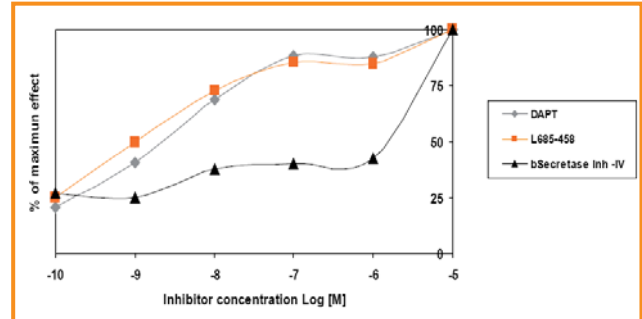
🔷 Determination of IC50 value

Determination of IC50 values for APP processing inhibitor LP226A1.
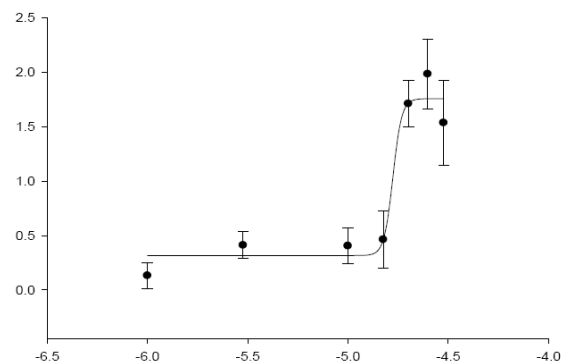

IC50 value for LP226A1 was determined by treating of MDCK APP model cells with inhibitor concentrations 30uM, 25uM, 20uM, 15uM, 10uM, 3 uM and 1uM during 72h. Followed this incubation, the intracellular vesicles retented are quantified. Error bars represent the standard deviation among 5 replicate wells. Ic50 for LP226A1 was 17,8uM and z' for this experiment was 0,90 +/-0,01.

🏃 Screening Campaign

Vesicles retention ratio of 1200 compounds screened at 10uM concentration. The positive control (LP226A1) is represented in gray. The negative control is represented in white color. Of the 1200 compound screened, 57 compounds exhibiting greater inhibition than our positive control.
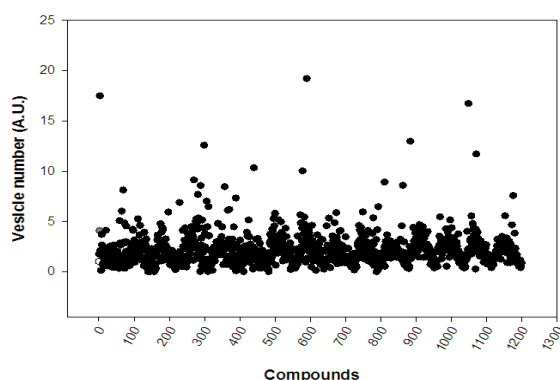
Z´values for 15 plates screened. Z´value media obtained was 0,62+/-0,13. In this assay, 14 plates had Z´factor grater than 0.5 and only one plate had a Z´factor less than 0,5 (was 0,47).
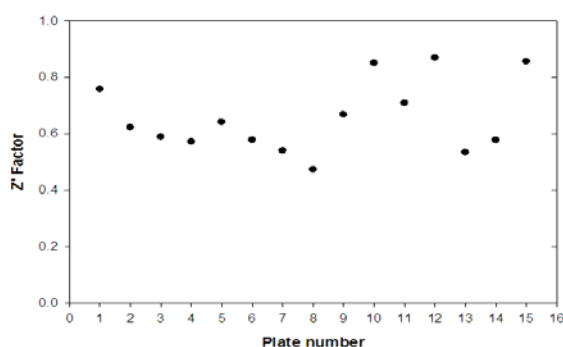
🏃 Applications

The Alzheimer model based in MDCK can be used in drug discovery for APP processing inhibitors o 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 compund effect in a multiparametric manner.

This model provide a strategy to evaluate drug agaits secretases activity without the necessity to be permeable..