Alzheimer disease (AD) is characterized by brain depositions of the beta amyloid (bA). The bA is the amyloid precursor protein (APP) digestion product, which is released from the cell after b-secretase and g-secretase proteolysis. A novel fluorescence-based assay of secretases activity for screening of new inhibitors has been developed. We have cloned the cDNA corresponding to APP wt and one APP truncated mutant in frame with tGFP protein localized in the C-terminal (TMAPP-tGFP). We have confirmed that APP wt, APP-tGFP and truncated mutant of APP-tGFP displayed the same cellular localization. After generation of stable expressing clones for the fluorescent deleted mutant in Madin-Darby canine kidney cells, we selected clones with different expression levels. After specific inhibition of b-secretase activity or g-secretase activity, the secretion pathway was blocked and the clone 6 adopted a vesicular pattern. This assay permits to evaluate the endogenous secretase proteolitc process in the space and time, using different inhibitory compounds to block the g-secretase activity, b-secretase or siRNA BACE to decrease its processing. High content analysis of b and g secretase activity was performed using an automated epifluorescent imaging system to acquire and analyze images and quantified the fluorescent vesicules into the citoplasm. The results indicated that the pharmacological inhibition of secretases implicated in AD remains a valid strategy for drug screening and these models are appropriate to monitorized the disease process in vivo in the multiparametric High Content Analyses.