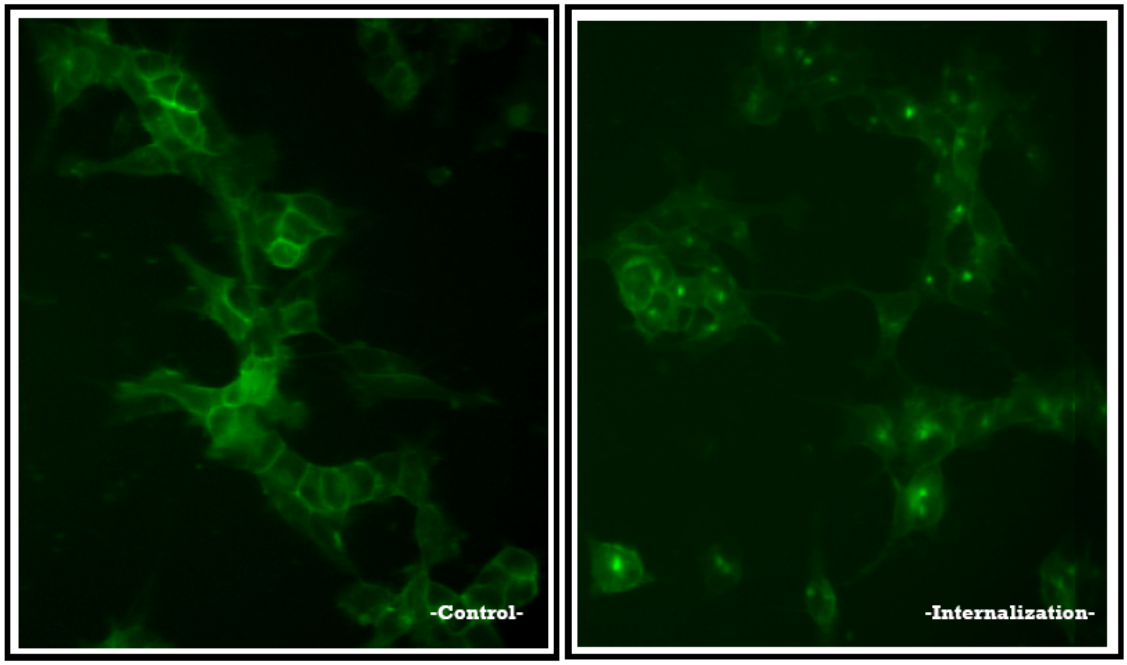
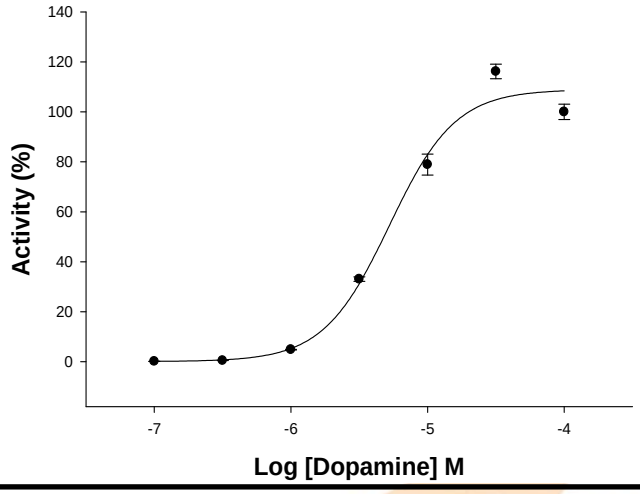
REF: P30218

RECEPTOR INTERNALIZATION ASSAYS

- FLUORESCENT HUMAN DOPAMINE RECEPTOR D1 CELL LINE -





Product name: DRD1-tGFP / SH-SY5Y cell line

Ec_{50} Dopamine: 5.25 x 10^{-6} M

Z´: 0.67+/- 0.02

VAT No. ESB95481909

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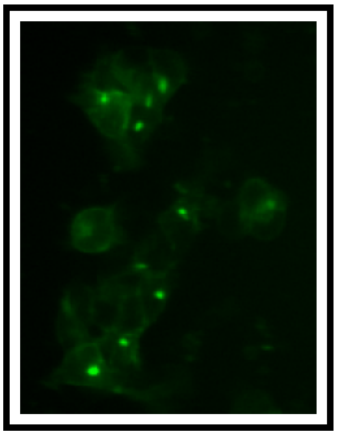

Product Name: DRD1-tGFP_SH-SY5Y

Reference: P30218

Recp. Official Full Name: Dopaminergic receptor D1 (DRD1)

DNA Accesion Number: Gene Bank NM_000794

Host Cell: SH-SY5Y

Resistance: G418

References:

- ***P30218:*** 2 vials of 3 x 10^6 proliferative cells
- ***P30218-DA***: 1 vial of 2 x 10^6 division-arrested cells

Storage: Liquid Nitrogen

Assay Briefly description

DRD1-tGFP_SH-SY5Y contains SH-SY5Y cells stably expressing human Dopaminergic Receptor D1 (DRD1) tagged in the N-terminus with tGFP protein.

Innoprot DRD1 redistribution Cell Line has been designed to assay potential agonists/ antagonists against DRD1, modulating Dopaminergic receptor D1 activation and the following redistribution process inside the cells. This cell line will allow the image analysis of the stimuli induced by the compounds.

This highly reproducible assay has been validated using Dopamine as a DRD1 agonist in a High Content Analysis (HCA).

About Dopaminergic Receptor D1

The gene encodes the D1 subtype of the Dopamine receptor. This subtype is a G-protein coupled receptor which stimulates adenylyl cyclase.

Dopamine is one of the most important neurotransmitters and the expression of its receptors is well characterized in brain.

Dopamine receptors are involved in many neurological processes so their abnormal signalling is implicated in several neuropsychiatric disorders.

DRD1 mediates some behavioural responses, regulates neuronal growth and development.

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Assay Characterization

Our expression plasmid containing the coding sequence of human Dopaminergic receptor D1 tagged in the N-terminal with tGFP protein. Our plasmid was transfected in SH-SY5Y cells. Resistant clones were obtained by limit dilution, and receptor gene expression was tested by RT-PCR (Fig.1).

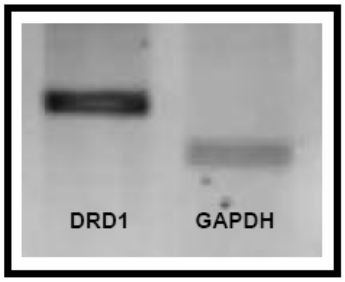

Fig1. DRD1 and GAPDH housekeeping gene RT-PCR.

Activation and Internalization assay for DRD1-tGFP (Ec50 =5.25 x 10^{-6}M)

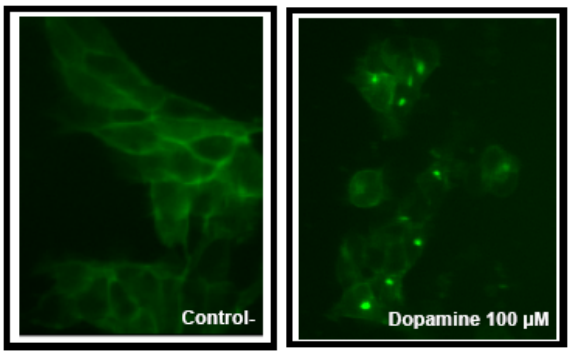

Fig2. Internalization of DRD1 stimulated with Dopamine. Concentrations from 0 to 100 µM were tested for 1h. Activation and internalization processes were detected and analyzed using "BD Pathway 855" High-Content Bioimager from BD Biosciences.

Assay Details

SH-SY5Y cells, stably expressing human Dopaminergic receptor D1 tagged in the N-terminus with tGFP protein, were stimulated with increasing concentrations of Dopamine during 1h. After the treatment, the fluorescent protein was internalized in vesicles in the cytosol; especially a big vesicle appeared next to the nucleus. Nuclei were stained with DAPI and Dopaminergic receptor D1 fluorescence redistribution was determined measuring the generation of the vesicle using image analysis algorithms.

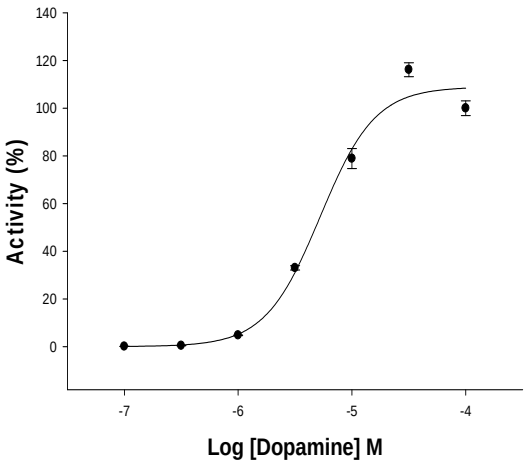

Fig3. Concentration response curve for Dopamine in Dopamine D1 receptor cell line. Cells were treated with 7 log dilution series (n=8). The Ec50 for the Dopamine was ~ 5.25x10^{-6}M after a treatment of 1 h with the agonist. Cells were fixed and the nuclei were stained with DAPI. % Activity was calculated relative to positive (100 µM). The internalization assay was validated with an average of Z´=0.67+/- 0.02 for High Content Screening.

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