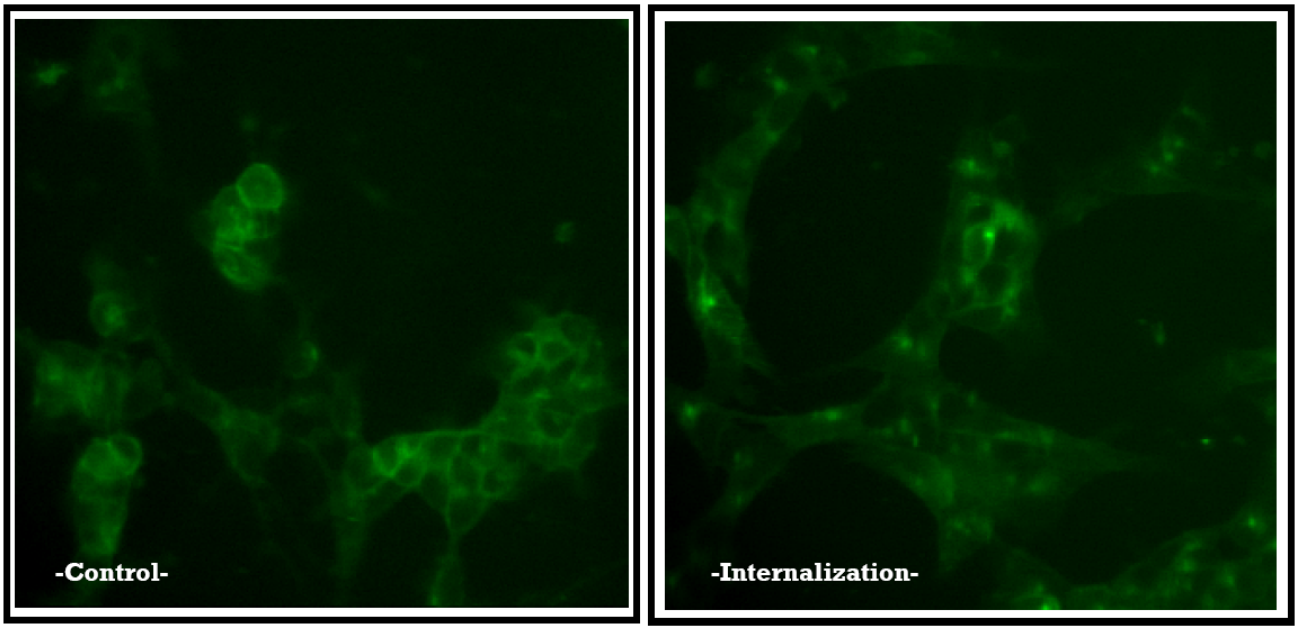
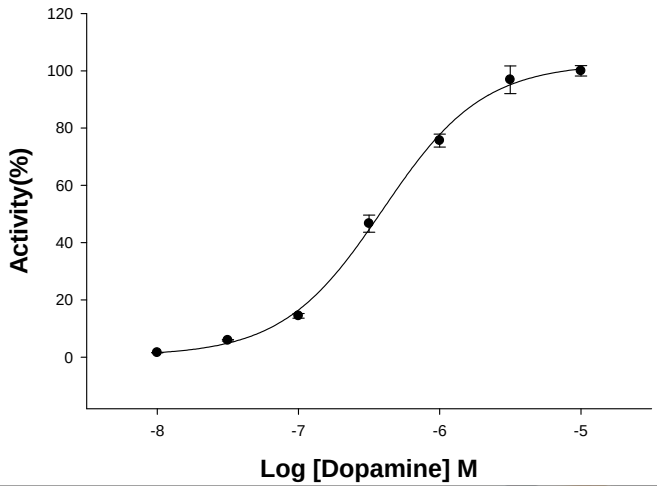
REF: P30220

RECEPTOR INTERNALIZATION ASSAYS

- FLUORESCENT HUMAN DOPAMINE RECEPTOR D3 CELL LINE -





Product name: DRD3-tGFP / SH-SY5Y cell line

Ec_{50} Dopamine: 3.9 x 10^{-7}M

Z´: 0.79+/- 0.02

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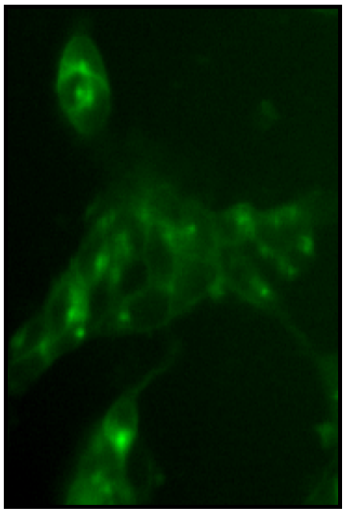

Product Name: DRD3-tGFP_SH-SY5Y

Reference: P30220

Recp. Official Full Name: Dopaminergic receptor D3 (DRD3)

DNA Accesion Number: Gene Bank NM_000796

Host Cell: SH-SY5Y

Resistance: G418

References:

- ***P30220:*** 2 vials of 3 x 10^6 proliferative cells
- ***P30220-DA***: 1 vial of 2 x 10^6 division-arrested cells

Storage: Liquid Nitrogen

Assay Briefly description

DRD3-tGFP_SH-SY5Y contains SH-SY5Y cells stably expressing human Dopaminergic Receptor D3 (DRD3) tagged in the N-terminus with tGFP protein.

Innoprot DRD3 redistribution Cell Line has been designed to assay potential agonists/ antagonists against DRD3, modulating Dopaminergic receptor D3 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 DRD3 agonist in a High Content Analysis (HCA).

About Dopaminergic Receptor D3

The gene encodes the D3 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.

DRD3 is found in the limbic areas of the brain, which usually are associated with cognitive, emotional, and endocrine functions. DRD3 is a target for drugs which treat schizophrenia, drug addiction, and Parkinson's disease.

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Assay Characterization

Our expression plasmid containing the coding sequence of human Dopaminergic receptor D3 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).

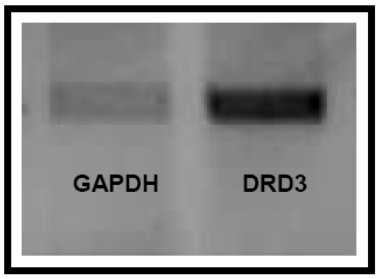

Fig1. GAPDH housekeeping gene and DRD3 **RT-PCR.**

Activation and Internalization assay for DRD3-tGFP (Ec50 = 3.9 x 10^{-7}M)

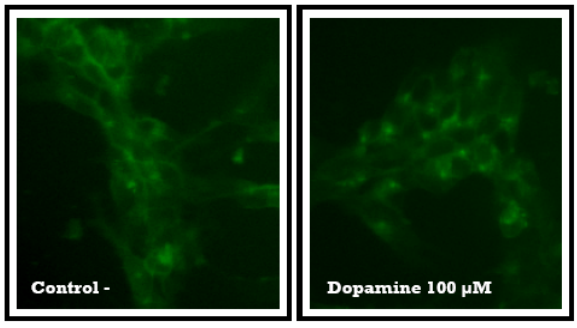

Fig2. Internalization of DRD3 stimulated with Dopamine. Concentrations from 0 to 100 µM were tested for 1h. Activation and internalization processes were detected and analyzed using "BD Pathway 855" High-Content Bioimager from BD Biosciences.

Assay Details

SH-SY5Y cells, stably expressing human Dopaminergic receptor D3 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 D3 fluorescence redistribution was determined measuring the generation of the vesicle using image analysis algorithms.

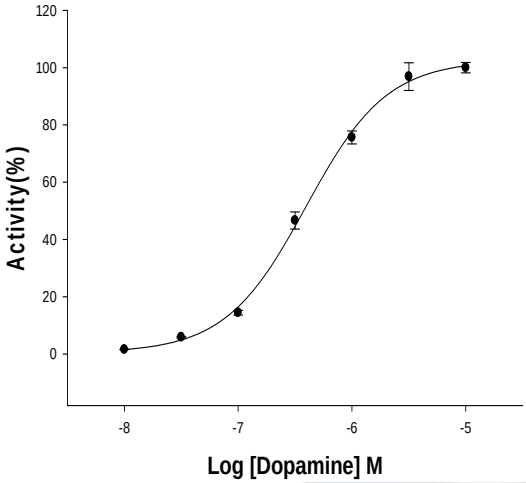

Fig3. Concentration response curve for Dopamine in Dopamine D3 receptor cell line. Cells were treated with 7 log dilution series (n=8). The Ec50 for the Dopamine was ~ 3.9x10^{-7} M after a treatment of 1 h with the agonist. Cells were fixed and the nuclei were stained with DAPI. % Activity was calculated relative to positive (100 µM). The internalization assay was validated with an average of Z´=0.79+/- 0.02 for High Content Screening.

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