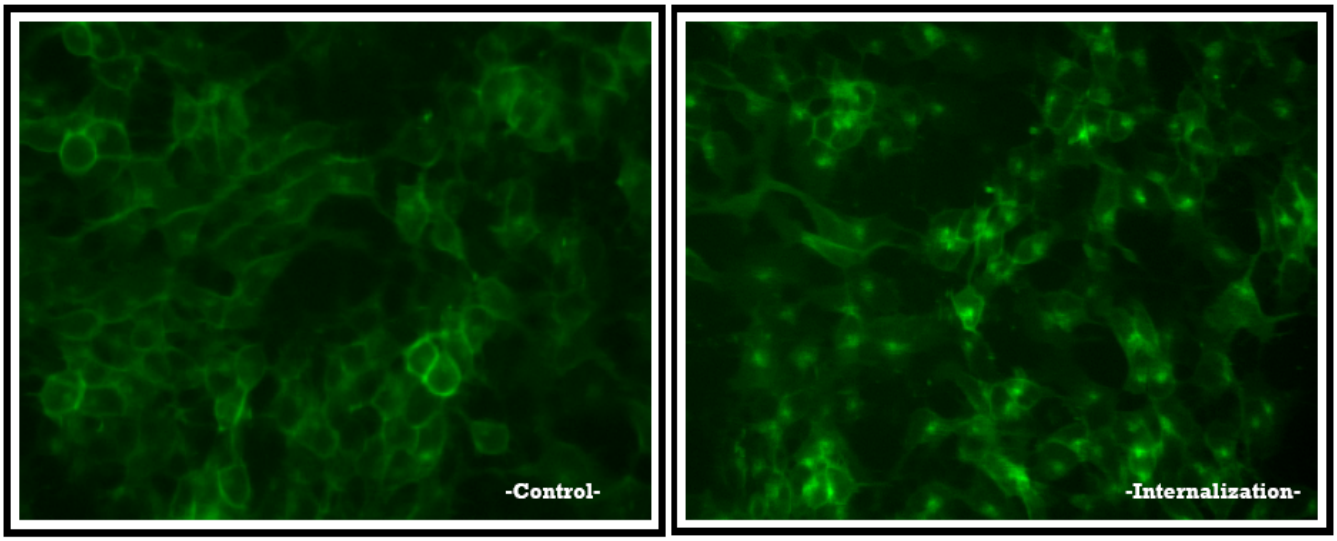
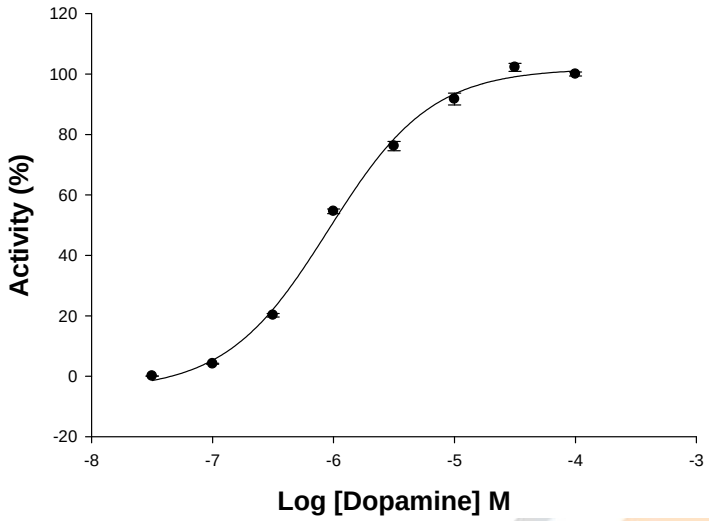
REF: P30222

# RECEPTOR INTERNALIZATION ASSAYS

## - FLUORESCENT HUMAN DOPAMINE RECEPTOR D5 CELL LINE -




**Product name:** DRD5-tGFP / SH-SY5Y cell line

**$Ec_{50}$ Dopamine:** 9.12 x $10^{-7}$ M

**Z´:** 0.81+/- 0.02

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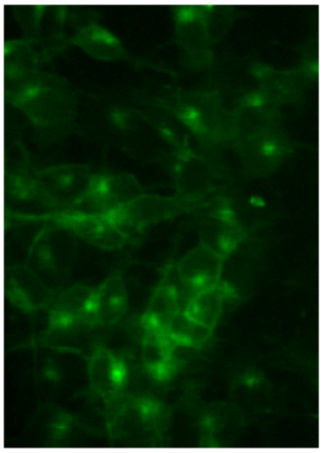

**Product Name:** DRD5-tGFP_SH-SY5Y

**Recp. Official Full Name:** Dopaminergic receptor D5 (DRD5)

**DNA Accesion Number:** GenBank: BC009748

**Host Cell:** SH-SY5Y

**Resistance:** G418

**References:**

- ***P30222:*** 2 vials of 3 x $10^6$ proliferative cells
- ***P30222-DA***: 1 vial of 2 x $10^6$ division-arrested cells

**Storage:** Liquid Nitrogen

## *Assay Briefly description*

DRD5-tGFP_SH-SY5Y contains SH-SY5Y cells stably expressing human Dopaminergic Receptor D5 (DRD5) tagged in the N-terminus with tGFP.

Innoprot DRD5 redistribution Assay kit has been designed to assay compounds or analyze stimuli for their ability to modulate Dopaminergic receptor D5 activation and the following redistribution process inside the cells.

This highly reproducible assay allows monitoring Dopaminergic receptor D5 activation and redistribution process in High Content Analysis and fluorescence microscope applications.

## *About Dopaminergic Receptor D5*

The gene encodes the D5 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.

Defects in DRD5 are a cause of benign essential blepharospasm (BEB), a primary focal dystonia affecting the orbicularis oculi muscles.

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## Assay Characterization

Our expression plasmid containing the coding sequence of human Dopaminergic receptor D5 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).

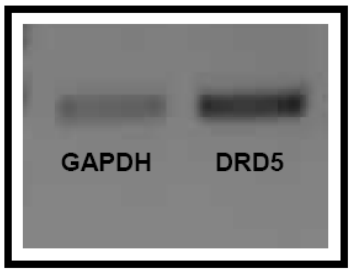


**Fig.1. GAPDH housekeeping gene and** DRD5 **RT-PCR.**

### Activation and Internalization assay for DRD5-tGFP (Ec50 = 9.12 x $10^{-7}$M)

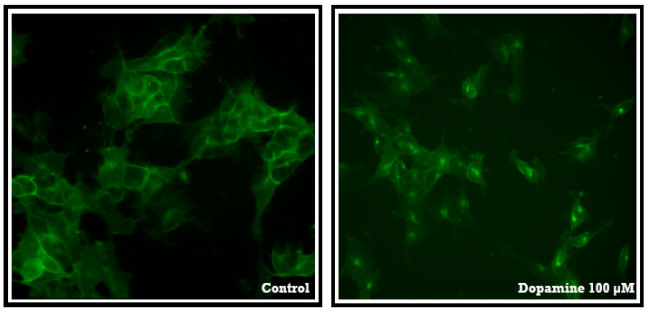


**Fig.2. Internalization of DRD5 stimulated with Dopamine.** Concentrations from 0 to 100 µM were tested for 1h. Activation and internalization processes were detected and analyzed using "BD Pathway 855" High-Content Bioimager from BD Biosciences.

## Assay Details

SH-SY5Y cells, stably expressing human Dopaminergic receptor D5 tagged in the N-terminus with tGFP, were stimulated with increasing concentrations of Dopamine during 1h. After the treatment the fluorescent protein was internalized in vesicles in the citosol, especially a big vesicle appeared next to the nucleus. Nuclei were stained with DAPI and Dopaminergic receptor D5 fluorescence redistribution was determined measuring the generation of the vesicle using image analysis algorithms.

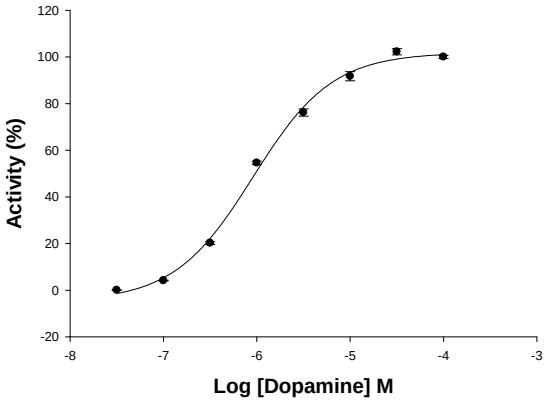


**Fig.3. Concentration response curve for Dopamine in Dopamine D5 receptor cell line.** Cells were treated with 8 log dilution series (n=8). The Ec50 for the Dopamine was ~ 9.12x$10^{-7}$ M after a treatment of 1 h with agonist. Cells were fixed and the nuclei were stained with DAPI. % Activity was calculated relative to positive (100 µM). The internalization assay was validated with an average of Z´=0.81+/- 0.02 for High Content Screening.

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