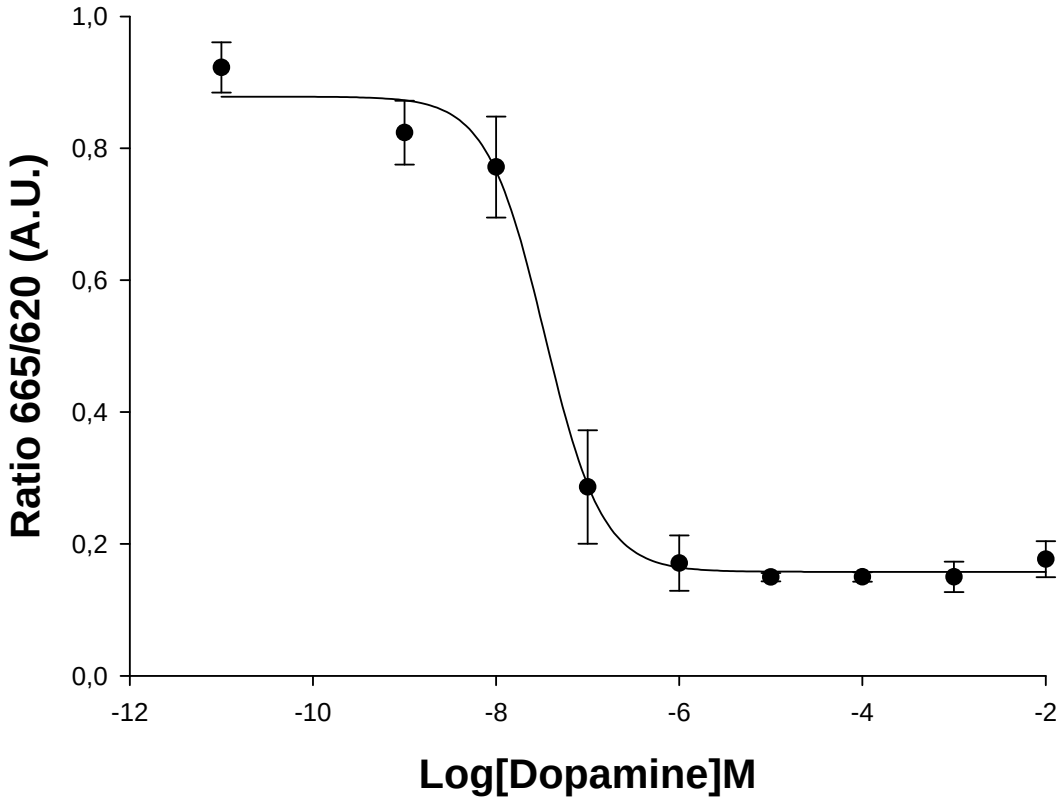
REF: P30118

HiTSeeker CELL LINES (LABEL-FREE GPCRS)

- DOPAMINE RECEPTOR D1 CELL LINE -



Product name: DRD1 (Dopamine receptor D1) /HEK293 cell line

Ec_{50} Dopamine: 3.38 x 10^{-8} M

Z´: 0.78+/- 0.02

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- DOPAMINE RECEPTOR D1 CELL LINE -

Product Name:	DRD1 /HEK293
Official Full Name:	Dopamine receptor D1
DNA Accesion Number:	NM_000794
Host Cell:	HEK293
Resistance:	Puromycin
References:	

- ***P30118:*** 2 vials of 3 x 10^6 proliferative cells
- ***P30118-DA***: 1 vial of 2.5 x 10^6 division-arrested cells

Storage: Liquid Nitrogen

Assay Briefly description

Each vial of HiTSeeker DRD1/HEK293 contains HEK293 cells stably expressing human dopamine receptor D1 with no tag.

Innoprot DRD1 cell line has been designed to assay compounds or analyze their capability to modulate dopamine receptor D1. When the agonist binds to DRD1 a G protein is activated, which in turn, triggers a cellular response mediated by second messengers (cAMP).

This cell line has been validated measuring cAMP increase in the cytosol. The high reproducibility of this assay allows monitoring ADRß3 activation process in High Throughput Screening.

About DRD1

This gene encodes the D1 subtype of the dopamine receptor. The D1 subtype is the most abundant dopamine receptor in the central nervous system. This G-protein-coupled receptor stimulates adenylyl cyclase and activates cyclic AMP-dependent protein kinases.

D1 receptors regulate neuronal growth and development, mediate some behavioral responses, and modulate dopamine receptor D2-mediated events. Alternate transcription initiation sites result in two transcript variants of this gene.

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Assay Characterization

Our expression plasmid contains the coding sequence of human DRD1 protein. Our plasmid was transfected in HEK293 cells. Resistant clones were obtained by limit dilution and receptor gene expression was tested by RT-PCR using GAPDH as internal control (Fig.1).

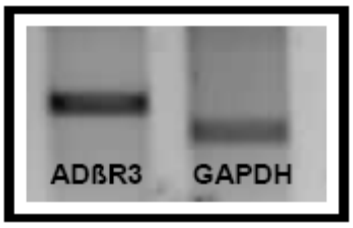

Fig.1. DRD1 and GAPDH housekeeping gene RT-PCR.

Validation of DRD1 cell line

cAMP production assay

cAMP production was assessed using the cAMP dynamic 2 kit (Cisbio). This kit contains labelled cAMP (620 nm) and an anti-cAMP antibody (665nm). Between these molecules occurs a fluorescence transfer (FRET). Native cAMP produced by cells (due to the binding of an agonist to its specific receptor) competes with the labelled cAMP producing a decrease of FRET detected by HTRF technology.

The specific signal is inversely proportional to the concentration of native cAMP produced by the binding of the agonist to its receptor.

Fluorescence detection was recorded in a Multi-Mode Microplate Reader Synergy 2 from Biotek.

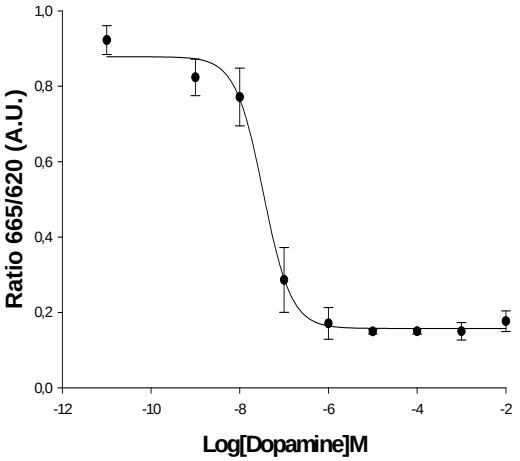

Fig.2. DRD1 dose response in AMP_c assay. Cells were treated with Dopamine concentrations ranging from 0 to 100 µM, n=3. The EC50 for Dopamine was ˜3.38x10-8M. The cAMP assay was validated with a Z´= 0.78+/- 0.02 for High Content Screening.

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