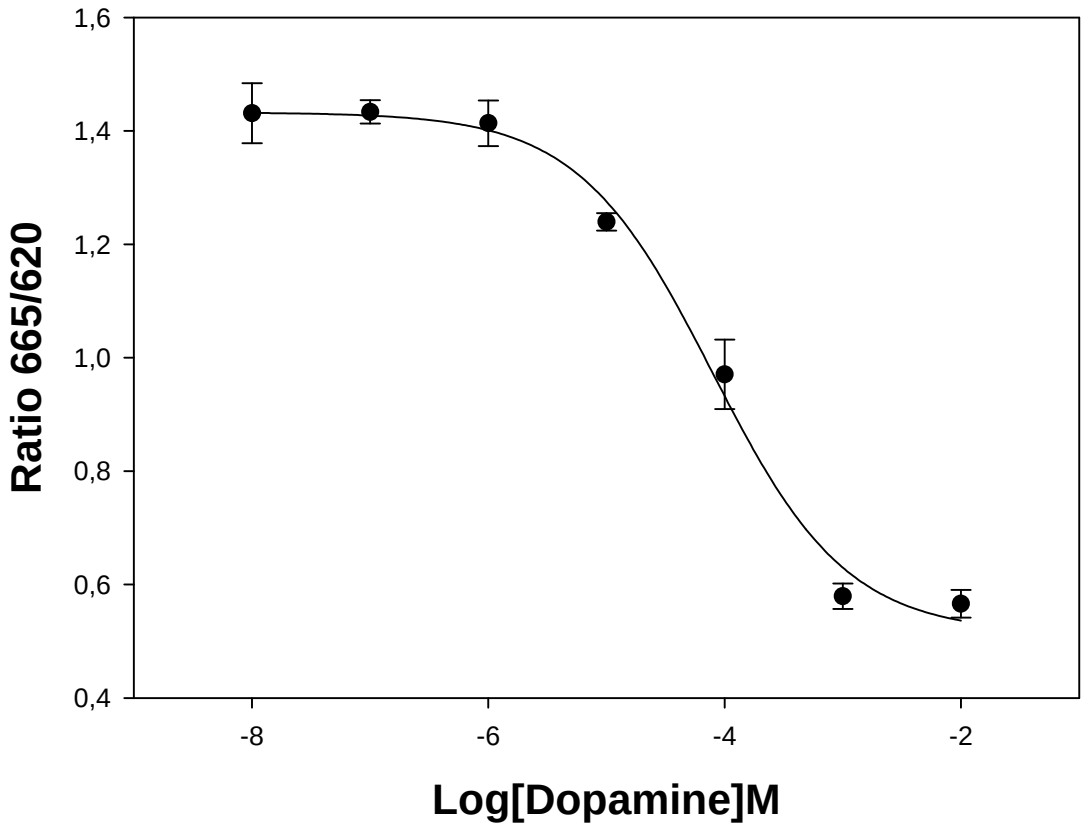
REF: P30122

HiTSeeker CELL LINES (LABEL-FREE GPCRS)

- DOPAMINE RECEPTOR D5 CELL LINE -



Product name: DRD5 (Dopamine receptor D5) /HEK293 cell line

Ec_{50} Dopamine: 7.91 x 10^{-5} M

Z´: 0.73+/- 0.02

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- DOPAMINE RECEPTOR D5 CELL LINE -

Product Name:	DRD5 / HEK293
Official Full Name:	Dopamine receptor D5
DNA Accesion Number:	NM_000798
Host Cell:	HEK293
Resistance:	Puromycin
References:	

- ***P30122:*** 2 vials of 3 x 10^6 proliferative cells
- ***P30122-DA***: 1 vial of 2.5 x 10^6 division-arrested cells

Storage: Liquid Nitrogen

Assay Briefly description

Each vial of HiTSeeker DRD5/HEK293 contains HEK293 cells stably expressing human dopamine receptor D5 with no tag.

Innoprot DRD5 cell line has been designed to assay compounds or analyze their capability to modulate dopamine receptor D5. When the agonist binds to DRD5 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 DRD5 activation process in High Throughput Screening.

About DRD5

Dopamine receptors are implicated in many neurological processes, including motivation, pleasure, cognition, memory, learning, and fine motor control, as well as modulation of neuroendocrine signaling. Abnormal dopamine receptor signaling and dopaminergic nerve function is implicated in several neuropsychiatric disorders. Thus, dopamine receptors are common neurologic drug targets.

The D_1 and D_5 receptors have a high degree of structural homology and few ligands are available that can distinguish between them as yet, however there are a number of ligands that are selective for $D_{1/5}$ over the other dopamine receptors. The recent development of a selective D_5 antagonist has allowed the action of D_1-mediated responses to be studied in the absence of a D_5 component, but no selective D_5 agonists are yet available.

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Assay Characterization

Our expression plasmid contains the coding sequence of human DRD5 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. DRD5 and GAPDH housekeeping gene RT-PCR.

Validation of DRD5 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.

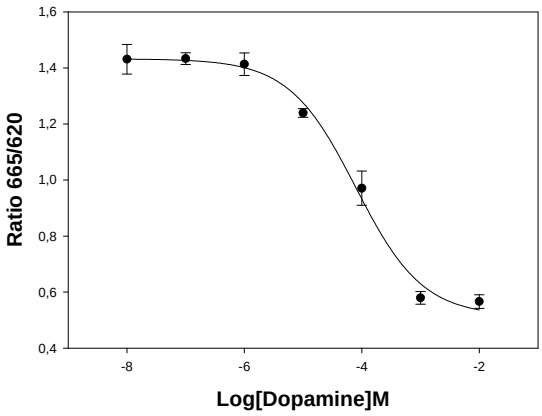

Fig.2. DRD5 dose response in AMP_c assay. Cells were treated with Dopamine concentrations ranging from 0 to 100 µM, n=3. The EC50 for Dopamine was ~3.38x10-8M. The cAMP assay was validated with a Z´= 0.78+/- 0.02 for High Content Screening.

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