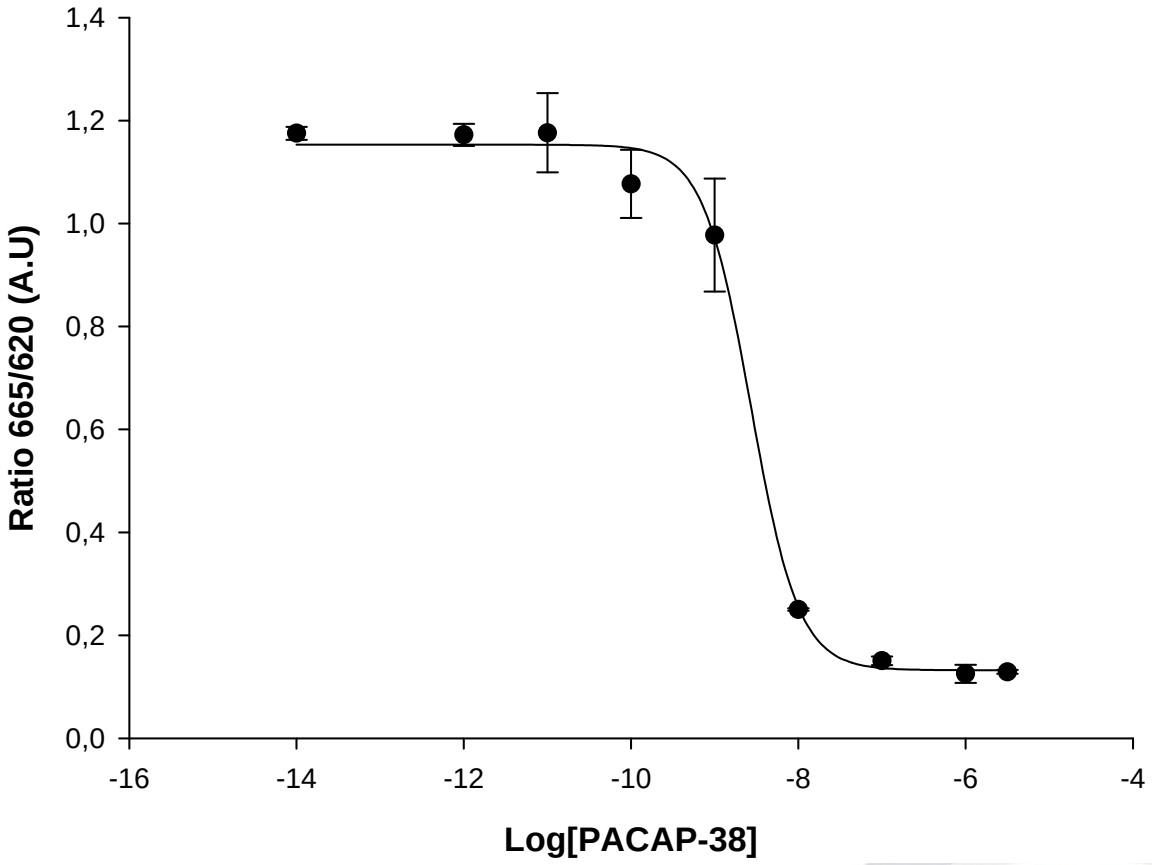
REF: P30112

HiTSeeker CELL LINES (LABEL-FREE GPCRS)

VASOACTIVE INTESTINALPOLYPEPTIDE RECEPTOR 1(VIPR1) CELL LINE -



Product name: VIPR1 /HEK293 cell line

Ec_{50} PACAP-38: $2.72x10^{-9}$M

Z´: 0.95 +/- 0.02

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- VASOACTIVE INTESTINALPOLYPEPTIDE RECEPTOR 1 -

Product Name:	VIPR1/HEK293
Official Full Name:	Vasoactive-intestinal-polypeptide receptor 1
DNA Accesion Number:	BC064424
Host Cell:	HEK293
Resistance:	Puromycin
References:	

- ***P30112:*** 2 vials of 3 x 10^6 proliferative cells
- ***P30112-DA***: 1 vial of 2.5 x 10^6 division-arrested cells

Storage: Liquid Nitrogen

Assay Briefly description

Each vial of HiTSeeker VIPR1 contains HEK293 cells stably expressing human Vasoactive intestinal polypeptide receptor 1 with no tag.

Innoprot's HiTSeeker VIPR1 cell line has been designed to assay compounds or analyze their capability to modulate Vasoactive intestinal polypeptide receptor. When the agonist binds to VIPR1 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 VIPR1 activation process in High Throughput Screening.

About VIPR1

The protein encoded by this gene belongs to the family of vasoactive intestinal polypeptide receptor. There are two known receptors for the vasoactive intestinal peptide (VIP) termed VPAC1 and VPAC2. VPAC1 is a receptor for vasoactive intestinal peptide (VIP), a small neuropeptide.

Vasoactive intestinal peptide is involved in smooth muscle relaxation, exocrine and endocrine secretion, and water and ion flux in lung and intestinal epithelia. Its actions are effected through integral membrane receptors associated with a guanine nucleotide binding protein which activates adenylate cyclase.

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Assay Characterization

Our expression plasmid contains the coding sequence of human VIPR1 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).

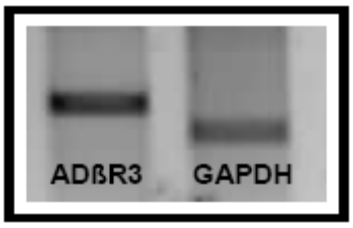

Fig.1. VIPR1 and GAPDH housekeeping gene RT-PCR.

Validation of VIPR1 cell line

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.

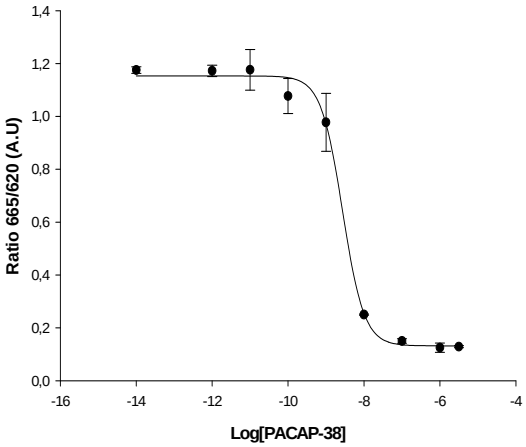

Fig.2.VIPR1 dose response AMPc production assay. Cells were treated with PACAP-38 concentrations ranging from 0 to 10 µM, n=3. The EC50 for PACAP-38 was ~ $2.72x10^{-9}$M. The cAMP assay was validated with a Z´= 0.953 for High Content Screening.

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