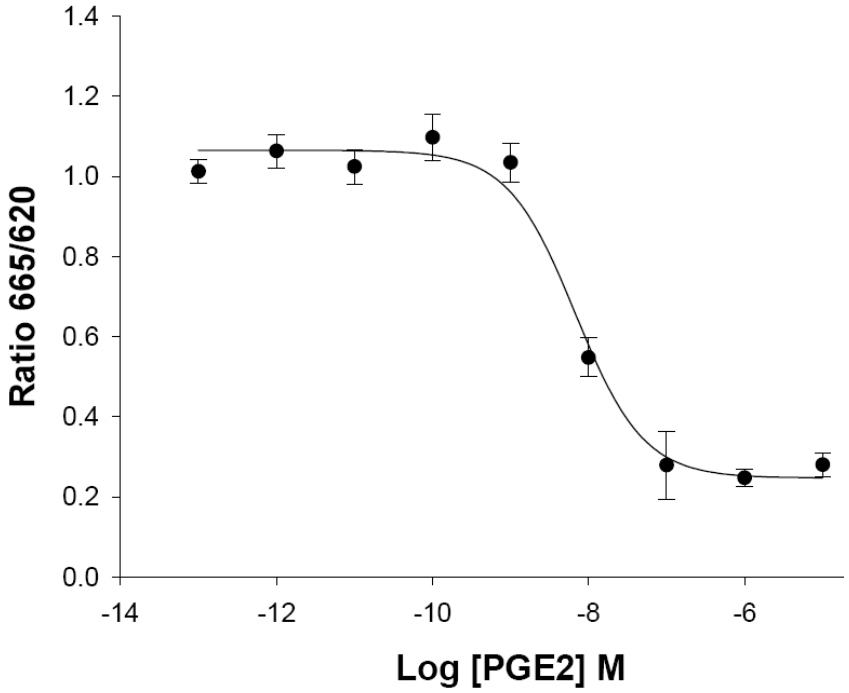
REF: P30405

HiTSeeker CELL LINES (LABEL-FREE GPCRS)

- PROSTAGLANDIN E RECEPTOR 4 (SUBTYPE EP4) CELL LINE



Product name: PTGER4-HEK293 cell line

Ec_{50} human Prostaglandin E2 (PGE 2) $6.83x10^{-9}$M

Z´: 0.76+/- 0.02

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- PROSTAGLANDIN E RECEPTOR 4 (SUBTYPE EP4) HEK293 CELL LINE -

Product Name:	PTGER4/HEK293
Official Full Name:	Prostaglandin E receptor 4 (subtype EP4)
DNA Accession Number:	GenBank: AY429109
Host Cell:	HEK293
Format:	2 cryopreserved vials
Resistance:	Puromycin
References:	
P30410	2 vials of 3 x 10^6 proliferative cells
P30410-DA	1 vial of 2.5 x 10^6 division-arrested cells
Storage:	Liquid Nitrogen

Assay Briefly description

Each vial of HiTSeeker PTGER4 contains HEK293 cells stably expressing human Prostaglandin E receptor 4 (PTGER4) with no tag.

Innoprot PTGER4 cell line has been designed to assay compounds or analyze their capability to modulate Prostaglandin E receptor 4 (PTGER4). When the agonist binds to PTGER4 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 PTGER4 activation process in High Throughput Screening.

About PTGER4

Prostaglandins are a group of lipid compounds that participate in a wide range of body functions.

The protein encoded by this gene is a member of the G-protein coupled receptor family. This protein is one of four receptors identified for prostaglandin E2 (PGE2).

PTGER4 encodes the prostaglandin receptor EP4 and it has been related with Crohn disease.

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Assay Characterization

Our expression plasmid contains the coding sequence of human PTGER4 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).

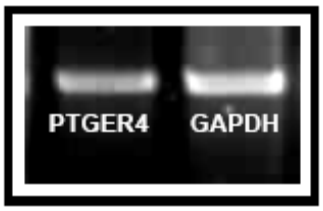

Fig.1.PTGER4 and GAPDH housekeeping gene RT-PCR.

Validation of PTGER4 cell line

cAMP production assay (Ec50=6.83x10^{-9}M)

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.

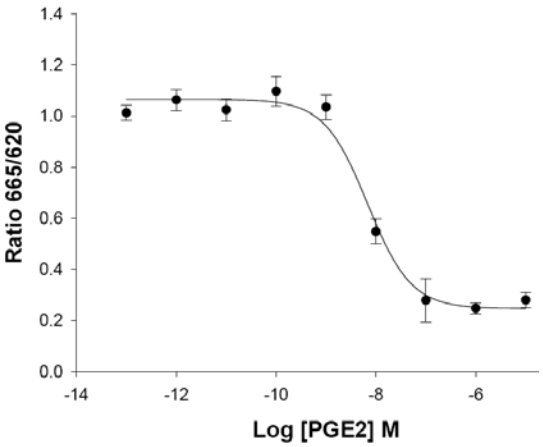

Fig.2. PGTER4 dose response in calcium assay. Cells were treated with PGE2 concentrations ranging from 0 to 10 μM, n=5. The EC50 for PGE2 ~6.83x10^{-9}M. The cAMP assay was validated with a Z´= 0.76+/- 0.02 for High Content Screening.

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