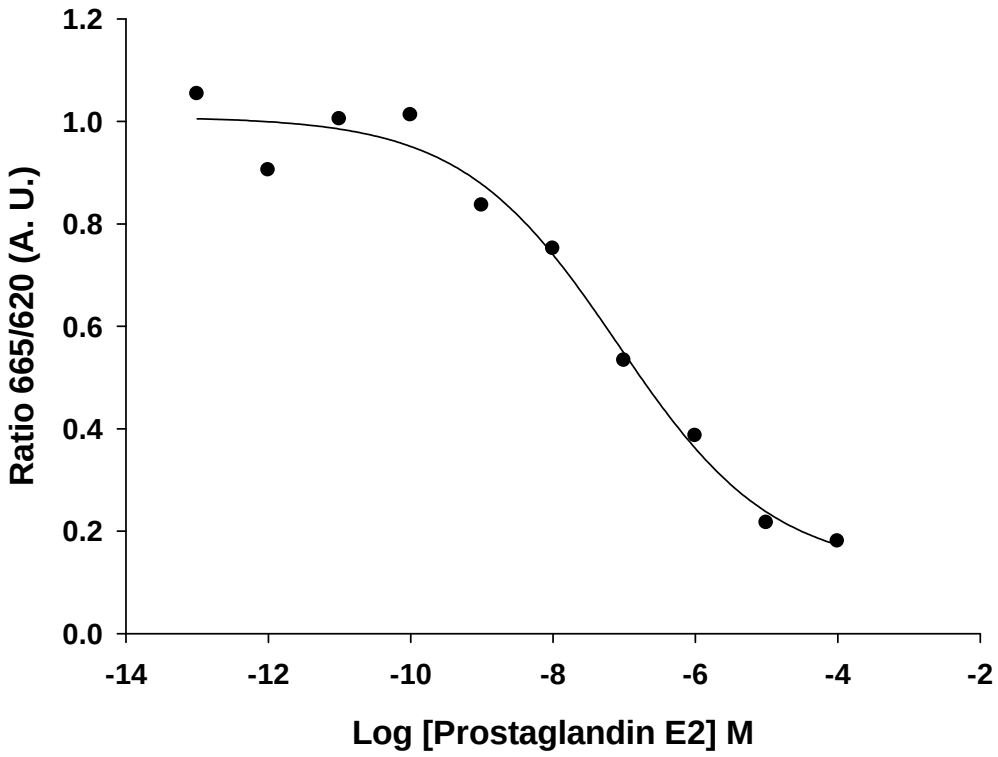
REF: P30403

HiTSeeker CELL LINES (LABEL-FREE GPCRS)

- PROSTAGLANDIN E RECEPTOR 2 (SUBTYPE EP2) CELL LINE -



Product name: PTGER2 /HEK293 cell line

Ec_{50} PGE2: 7.99x10^{-8} M

Z´: 0.73+/- 0.02

INNOVATIVE TECHNOLOGIES IN BIOLOGICAL SYSTEMS, S.L.
Parque Tecnológico Bizkaia, Edifício 502, 1ª Planta | 48160 | Derio | Bizkaia
Tel.: +34 944005355 | Fax: +34 946579925
innoprot@innoprot.com | www.innoprot.com

REF: P30403

- PROSTAGLANDIN E RECEPTOR 2 (SUBTYPE EP2) CELL LINE -

Product Name:	PTGER2/HEK293
Official Full Name:	Prostaglandin E receptor 2
DNA Accesion Number:	GenBank: AY275471
Host Cell:	HEK293
Format:	2 cryopreserved vials
Resistance:	Puromycin
References:	
P30403	2 vials of 3 x 10^6 proliferative cells
P30403-DA	1 vial of 2.5 x 10^6 division-arrested cells
Storage:	Liquid Nitrogen

Assay Briefly description

Each vial of HiTSeeker PTGER2 contains 3 million HEK293 cells stably expressing human Prostaglandin E Receptor 2 with no tag.

Innoprot HiTSeeker PTGER2 has been designed to assay compounds or analyze their capability to modulate Prostaglandin E Receptor 2. When the agonist binds to PTGER2, 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 PTGER2 activation process in High Throughput Screening.

About PTGER2

PTGER2, also known EP2, is part of the Prostanoid Receptors family.

The ligand of PTGER2 is the Prostaglandin E2. This is a metabolite of arachidonic acid and its production is mediated by the enzime cycloxigenase. Prostaglandin E2 has different biological activities in different tissues but most of them are related to inflammatory and anaphylactic reactions.

When Prostaglandin E2 binds to its receptor, a G-protein signal cascade activates adenylyl cyclase and intercellular levels of cAMP rise.

INNOVATIVE TECHNOLOGIES IN BIOLOGICAL SYSTEMS, S.L.
Parque Tecnológico Bizkaia, Edifício 502, 1ª Planta | 48160 | Derio | Bizkaia
Tel.: +34 944005355 | Fax: +34 946579925
innoprot@innoprot.com | www.innoprot.com

Assay Characterization

Our expression plasmid contains the coding sequence of human PTGER2 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).

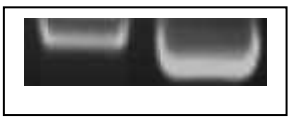


Fig.1. PTGER2 and GAPDH housekeeping gene RT-PCR.

Validation of PTGER2 cell line

cAMP production assay
(Ec50= 7.99x10^{-8} 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 (Fig. 2).

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

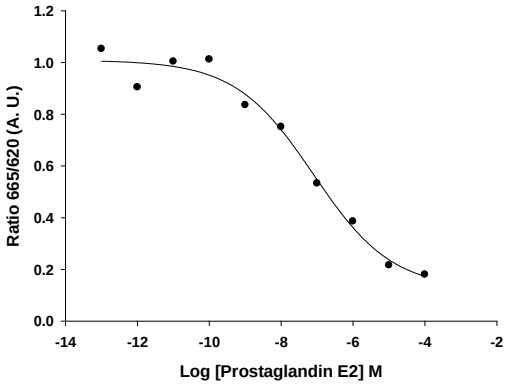


Fig.2. Prostaglandin E2 dose response in cAMP assay. Cells were treated with **Prostaglandin E2** concentrations ranging from 0 to 100 µM, n=3. The EC50 for **PGE2** was 7.99x10^{-8} M. The cAMP assay was validated with a Z´= 0.73+/- 0.02 for High Content Screening.

INNOVATIVE TECHNOLOGIES IN BIOLOGICAL SYSTEMS, S.L.
Parque Tecnológico Bizkaia, Edifício 502, 1ª Planta | 48160 | Derio | Bizkaia
Tel.: +34 944005355 | Fax: +34 946579925
innoprot@innoprot.com | www.innoprot.com