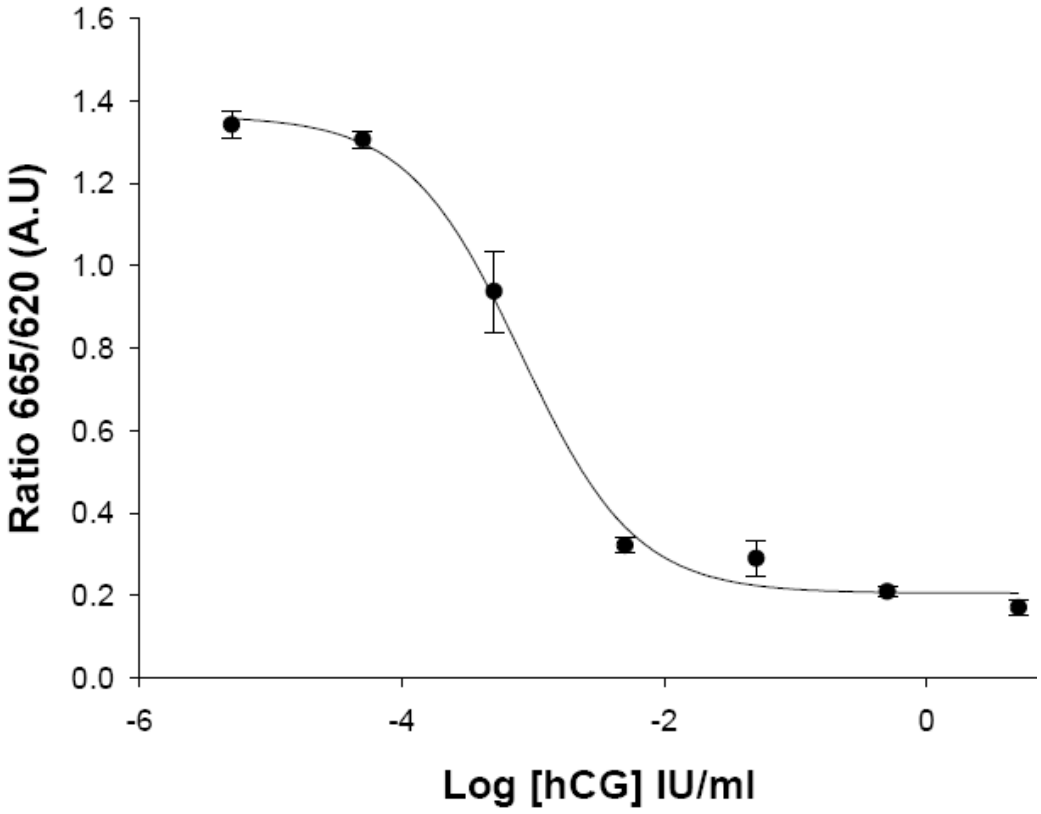
REF: P30177

HiTSeeker CELL LINES (LABEL-FREE GPCRS)

- LUTEINIZING HORMONE/CHORIOGONADOTROPIN REC. (LHCGR) CELL LINE -



Product name: LHR-LCGR /HEK293 cell line

Ec_{50} human Chorionic Gonadotropin (hCG): $8.08x10^{-4}$IU/ml

Z´: 0.75+/- 0.02

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- LUTEINIZING HORMONE/CHORIOGONADOTROPIN REC. HEK293 CELL LINE -

Product Name:	LHCGR/HEK293
Official Full Name:	Luteinizing hormone/choriogonadotropin receptor
DNA Accession Number:	GenBank: BC156303 / NM_000233
Host Cell:	HEK293
Format:	2 cryopreserved vials
Resistance:	Puromycin
References:	

- ***P30177:*** 2 vials of 3 x 10^6 proliferative cells
- ***P30177-DA***: 1 vial of 2.5 x 10^6 division-arrested cells

Storage:	Liquid Nitrogen

Assay Briefly description

Each vial of HiTSeeker LHCGR contains HEK293 cells stably expressing human Luteinizing hormone receptor (LHR) / choriogonadotropin receptor (LCGR) with no tag.

Innoprot's HiTSeeker LHCGR cell line has been designed to assay compounds or analyze their capability to modulate Luteinizing hormone / choriogonadotropin receptor (LHCGR). When the agonist binds to LHCGR a G protein is activated, which in turn, triggers a cellular response mediated by second messengers (cAMP).

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

About LHCGR

The **luteinizing hormone / choriogonadotropin receptor** (**LHCGR**), is a transmembrane receptor found in the ovary and testis but also in placent or uterus.

The receptor interacts with both luteinizing hormone (LH) and chorionic gonadotropins (such as hCG in humans) and represents a G protein-coupled receptor (GPCR) that activates cAMP system.

In the ovary, the LHCG receptor is required for follicular maturation, ovulation and luteal function. Its expression requires appropriate hormonal stimulation by FSH and estradiol.

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Assay Characterization

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

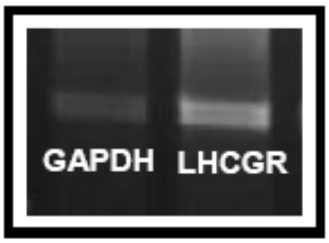

Fig.1. GAPDH housekeeping gene and LHCGR RT-PCR.

Validation of LHCGR cell line

cAMP production assay (Ec50=8.08x10^{-4}IU/ml)

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.

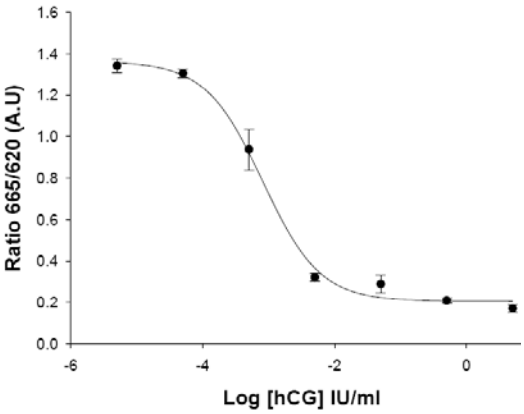

Fig.2. LHCGR **dose response in calcium assay.** Cells were treated with **hCG (human Chorionic Gonadotropin)** concentrations ranging from 0 to 5 IU/ml, n=5. The EC50 for **hCG** was **~8.08x10^{-4}IU/ml**. The cAMP assay was validated with a Z´= 0.75+/- 0.02 for High Content Screening.

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