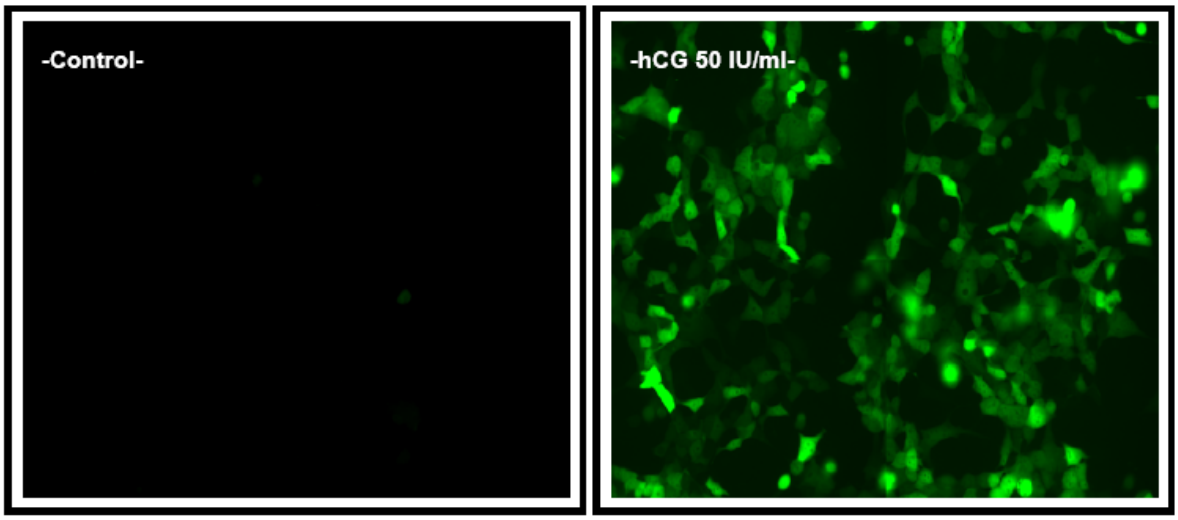
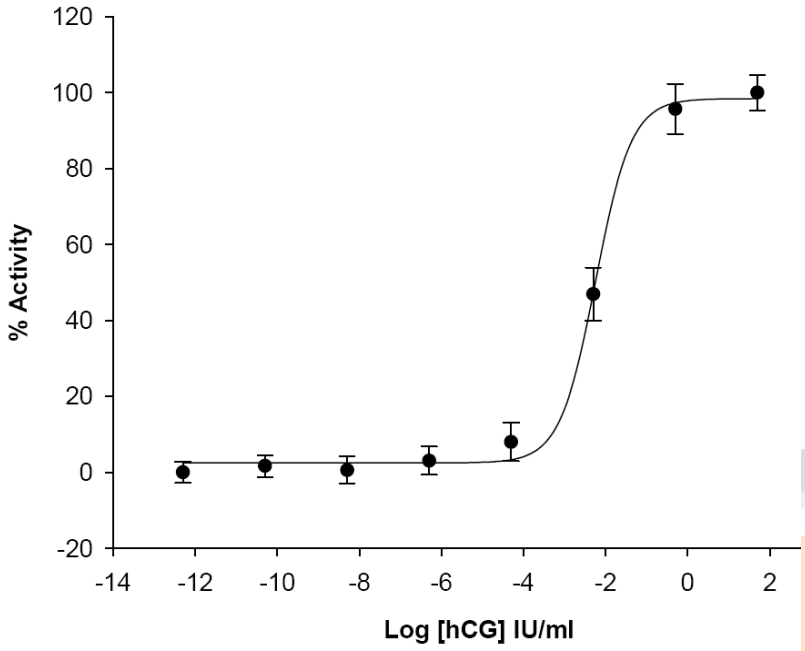
REF: P30177-F

FSHR REPORTER CELL LINES (FLUO-HiTSeeker Cell Lines)

LUTEINIZING HORMONE/CHORIOGONADOTROPIN RECEPTOR (LHCGR)





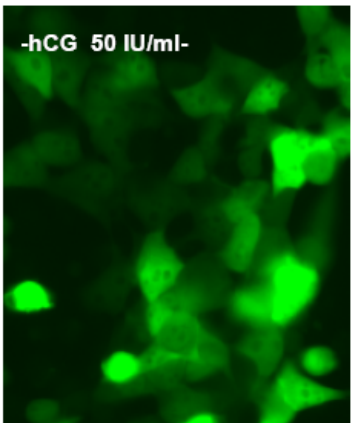
Product name: LHCGR-CRE-tGFP / HEK293 cell line

Ec_{50} hCGhuman: $5.76x10^{-3}$ IU/ml

Z´: 0.78+/- 0.02

VAT No. ESB95481909

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



Product Name: LHCGR /HEK293

Reference: P30177-F

Rep. Official Full Name: Luteinizing hormone/choriogonadotropin receptor

DNA Accession Number: Gene Bank BC156303 / NM_000233

Host Cell: HEK293

Resistance: Hygromycin/Puromycin

Quantity: > 3 x 10^6 cells / vial

Storage: Liquid Nitrogen

Assay Briefly description

FluoHiTSeeker LHCGR stable cell line contains HEK293 cells stably expressing human Luteinizing hormone / choriogonadotropin receptor (LHCGR) with no tag, and CRE-tGFP reporter construction.

Innoprot FluoHiTSeeker LHCGR cell line has been designed to assay compounds or analyze their capability to modulate Luteinizing hormone / choriogonadotropin receptor. 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 tGFP fluorescence production driven by a sensitive cAMP response element (CRE).The high reproducibility of this assay allows monitoring LHCGR receptor 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.

VAT No. ESB95481909

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 LHCG receptor 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).

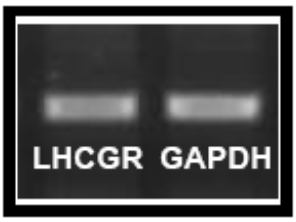


Fig1.LHCGR and GAPDH housekeeping gene RT-PCR.

Validation of LHCGR cell line

cAMP production assay (Ec50= 5.76x10^{-3} IU/ml)

cAMP production was assessed measuring the increase of fluorescence after the treatment with the agonist (hCG).

The specific signal is proportional to the concentration of native cAMP produced by the binding of the agonist to its receptor.

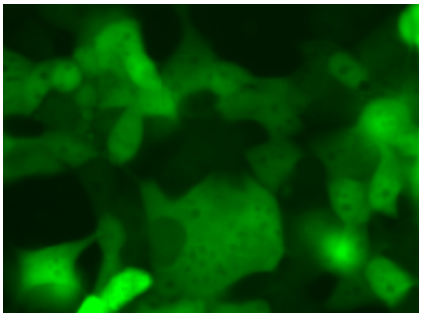


Fig2. Fluorescence increase after 24 h of treatment with the agonist.

Assay Details

HEK293 cells, stably expressing human LHCGR cotransfected with a CRE-tGFP construction, were stimulated with increasing concentrations of **human hCG during 24 h.** After the treatment an increase of fluorescence was observed. Fluorescence detection was recorded in a Multi-Mode Microplate Reader Synergy 2 from Biotek.

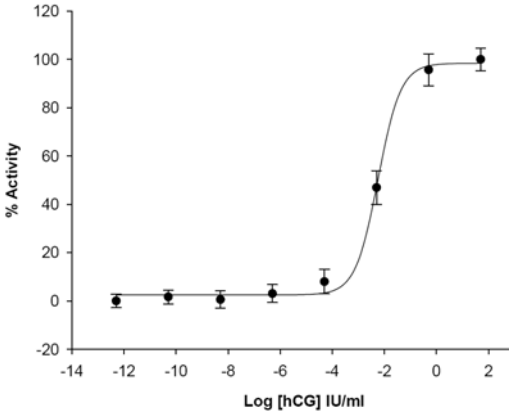


Fig3. LHCGR dose response curve in cAMP assay. Cells were treated with Human hCG. Concentrations from 0 to 50 IU/ml were tested (n=6). The Ec50 for the hCG is ~ **5.76x10^{-3}** IU/ml. The cAMP assay was validated with a Z´=0.78 for High Content Screening.

VAT No. ESB95481909
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