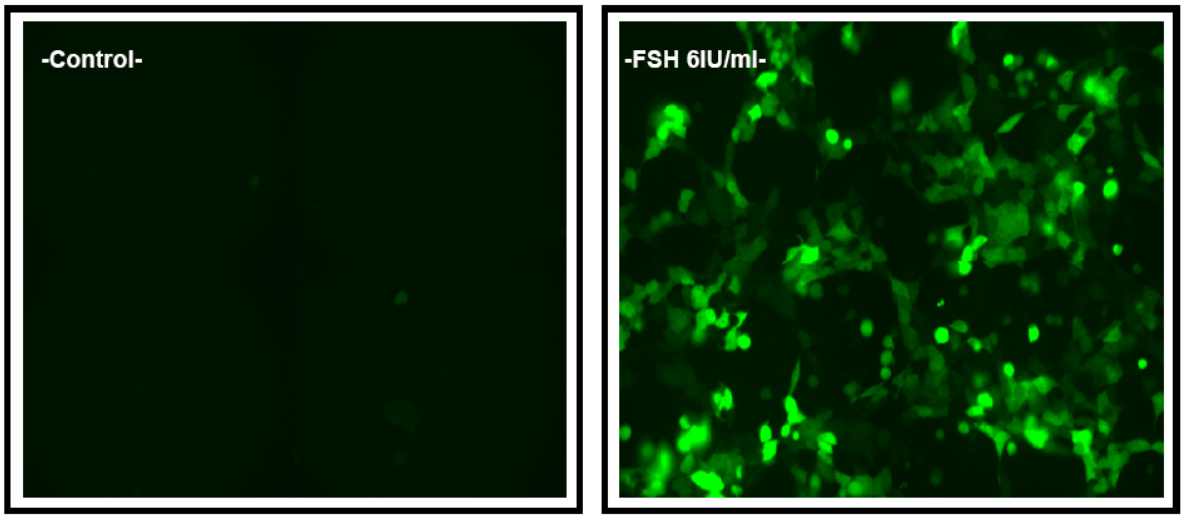
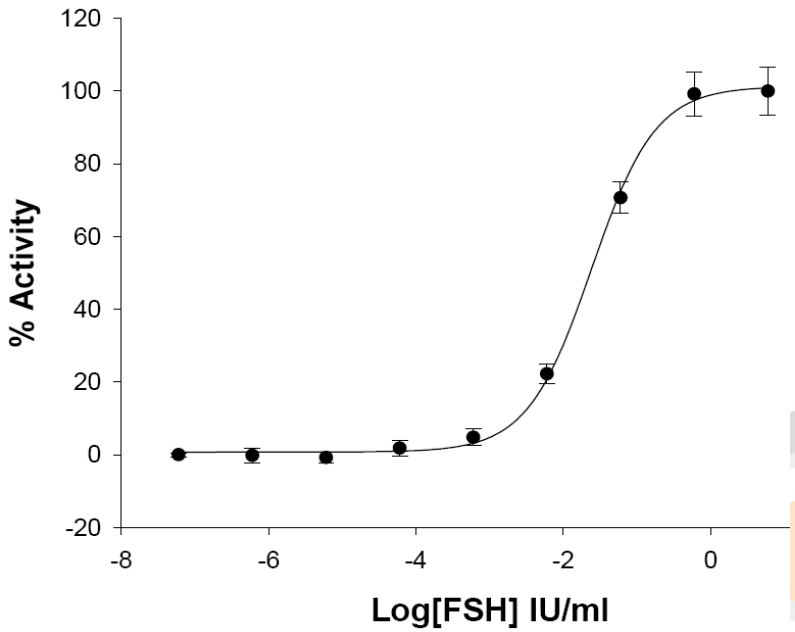
REF: P30117-F

FLUO-HitSeeker FSHR

HUMAN FOLLICLE STIMULATING HORMONE RECEPTOR REPORTER CELL LINE





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

Ec_{50} FSHhuman: 0.024 IU/ml

Z´: 0.78+/- 0.02

VAT No. ESB95481909

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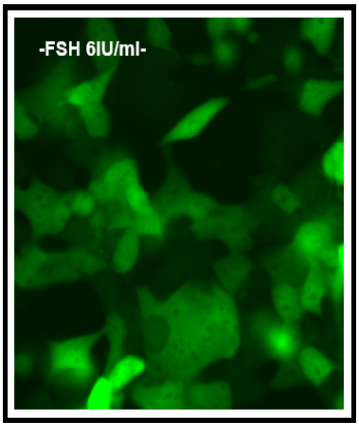

Product Name: Fluo-HiTSeeker FSHR /HEK293

Reference: P30117-F

Rep. Official Full Name: Follicle Stimulating Horm. Recpt.

DNA Accession Number: Gene Bank AY429104

Host Cell: HEK293

Resistance: Hygromycin/Puromycin

Quantity: > 3 x 10^6 cells / vial

Storage: Liquid Nitrogen

Assay Briefly description

FSHR/HEK293 contains HEK293 cells stably expressing human Follicle Stimulating Hormone Receptor (FSHR) with no tag, and CRE-tGFP reporter construction.

Innoprot FSHR cell line has been designed to assay compounds or analyze their capability to modulate Follicle stimulating hormone receptor. When the agonist binds to FSHR 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 FSHR receptor activation process in High Throughput Screening.

About FSHR

Follicle stimulating hormone receptor belongs to a family of G-protein coupled receptors which activate adenylate cyclase.

FSHR is a transmembrane receptor that interacts with the follicle stimulating hormone (FSH).

In the ovary, the FSH receptor is necessary for follicular development and it is expressed on the granulosa cells. In the male, the FSH receptor has been identified on the Sertoli cells that are critical for spermatogenesis. Mutations in this gene cause ovarian dysgenesis type 1, and also ovarian hyperstimulation syndrome.

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Assay Characterization

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

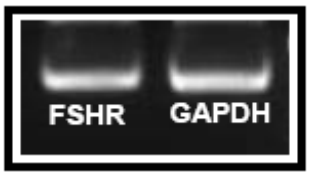

Fig1.FSHR and GAPDH housekeeping gene RT-PCR.

Validation of FSHR cell line

cAMP production assay (Ec50= 0.024 IU/ml)

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

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

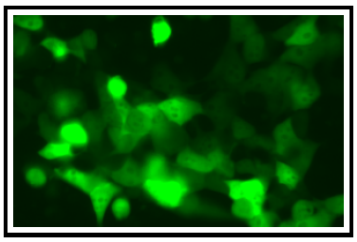

Fig2. Fluorescence increase after 24 h of treatment with the agonist.

Assay Details

HEK293 cells, stably expressing human FSHR cotransfected with a CRE-tGFP construction, were stimulated with increasing concentrations of **human FSH 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.

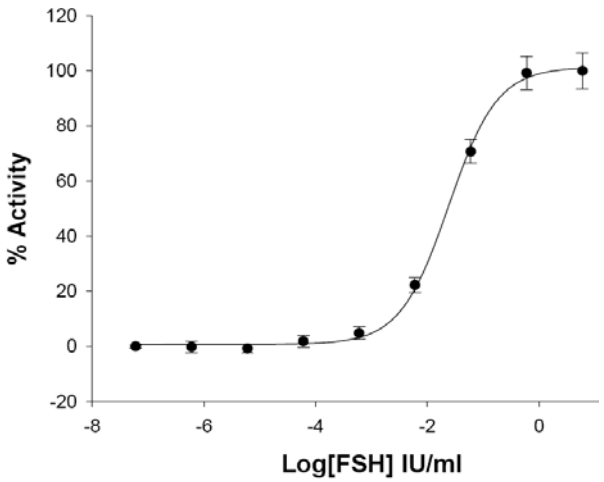

Fig3. FSH dose response curve in cAMP assay. Cells were treated with Human FSH (from urine of post-menopausal women). Concentrations from 0 to 6 IU/ml were tested (n=6). The Ec50 for the FSH is ~ 0.024 IU/ml. The cAMP assay was validated with a Z´=0.78 for High Content Screening.

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