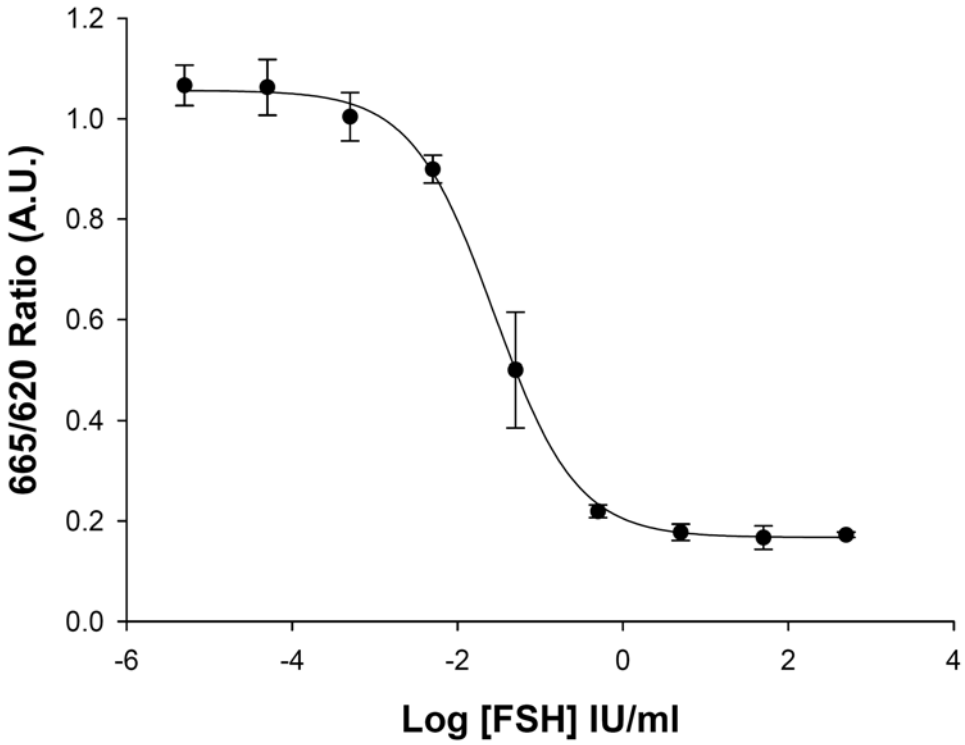
REF: P30117

HiTSeeker CELL LINES (LABEL-FREE GPCRS)

- HUMAN FOLLICLE STIMULATING HORMONE RECEPTOR HEK293 CELL LINE -



Product name: FSHR / HEK293 cell line

Ec_{50} FSH: 0.02 IU/ml

Z´: 0.85 +/- 0.02

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HiTSeeker CELL LINES (LABEL-FREE GPCRS)

HUMAN FOLLICLE STIMULATING HORMONE RECEPTOR CELL LINE

Product Name:	FSHR /HEK293
Official Full Name:	Follicle Stimulating Hormone Receptor
DNA Accesion Number:	GenBank: AY429104
Host Cell:	HEK293
Format:	Cryopreserved vials
Resistance:	G418 (Geneticin)
Size:	*P30117*: 2 vials of 3 x 10^6 proliferative cells *P30117-DA*: 1 vial of 2.5x10^6 division-arrested cells
Storage:	Liquid Nitrogen

Assay Briefly description

Each vial of HiTSeeker FSHR contains HEK293 cells stably expressing human Follicle Stimulating Hormone Receptor (FSHR) with no tag.

HiTSeeker 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 cAMP increase in the cytosol. The high reproducibility of this assay allows monitoring FSH 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).

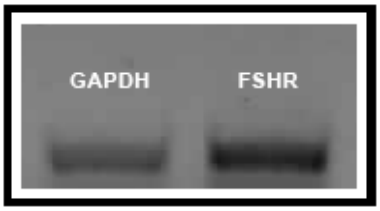

Fig.1. FSHR and GAPDH housekeeping gene RT-PCR.

Validation of FSHR cell line

cAMP production assay (Ec50= 0.02 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.

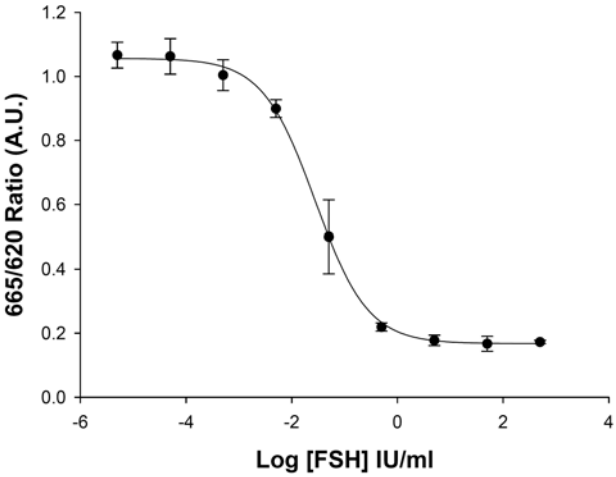

Fig.2. FSH dose response curve in cAMP assay. Cells obtained from batch number 230211 (clone 17) were treated with Human FSH (from urine of post-menopausal women). Concentrations from 0 to 500 IU/ml were tested by quadruplicate. The Ec50 for the FSH is ~ 0.02 IU/ml. The cAMP assay was validated with a **Z´=0.85** for High Content Screening.

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