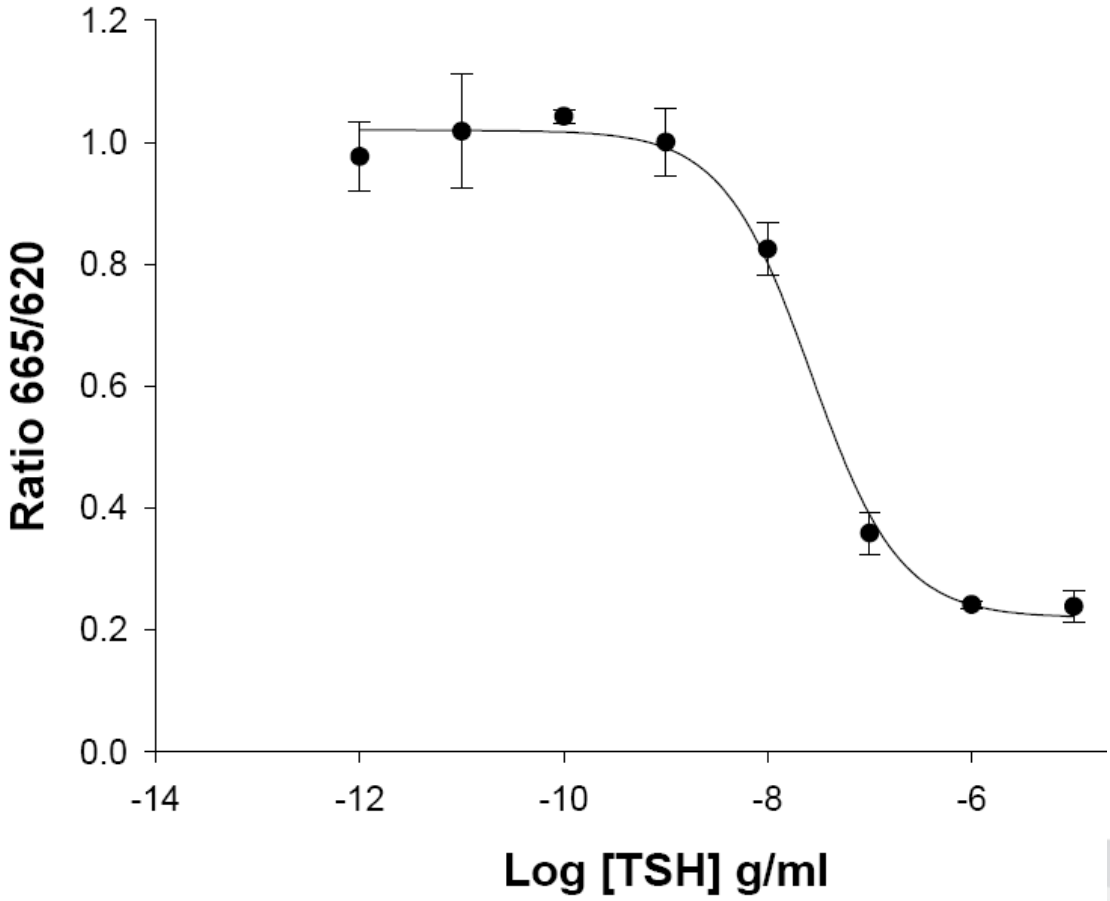
REF: P30199

HiTSeeker CELL LINES (LABEL-FREE GPCRS)

- THYROID STIMULATING HORMONE RECEPTOR CELL LINE -



Product name: TSHR /HEK293 cell line

Ec_{50} TSH: $2.67x10^{-8}$gr/ml

Z´: 0.76+/- 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

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- THYROID STIMULATING HORMONE RECEPTOR CELL LINE -

Product Name:	TSHR/HEK293
Official Full Name:	Thyroid stimulating hormone receptor
DNA Accesion Number:	GenBank: AY429111
Host Cell:	HEK293
Resistance:	G418
References:	

- ***P30199*** 2 vials of 3 x 10^6 proliferative cells
- ***P30199-DA***: 1 vial of 2.5 x 10^6 division-arrested cells

Storage: Liquid Nitrogen

Assay Briefly description

TSHR/HEK293 contains HEK293 cells stably expressing human Thyroid Stimulating Hormone Receptor with no tag.

Innoprot TSHR cell line has been designed to assay compounds or analyze their capability to modulate Thyrois Stimulating Hormone Receptor. When the agonist binds to TSHR 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 TSHR activation process in High Throughput Screening.

About TSHR

The ligand of TSHR is the Thyroid Stimulating Hormone. TSH or thyrotropin is a hormone synthesized in the anterior pituitary gland that stimulates the thyroid gland to secrete the hormones thyroxine (T4) and triiodothyronine (T3). It consists of an alpha subunit and a beta subunit.

TSH production is regulated by hormones secreted by hypothalamus like Thyrotropin Releasing Hormone (TRH) or Somatostatin. The levels of Thyroid hormones T3 and T4, in turn, have a regulatory feedback effect on TSH.

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

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Assay Characterization

Our expression plasmid contains the coding sequence of human TSHR 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. TSHR and GAPDH housekeeping gene RT-PCR.

Validation of TSHR cell line

cAMP production assay
(Ec50= 1.62x10^{-7}gr/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 (Fig. 2).

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

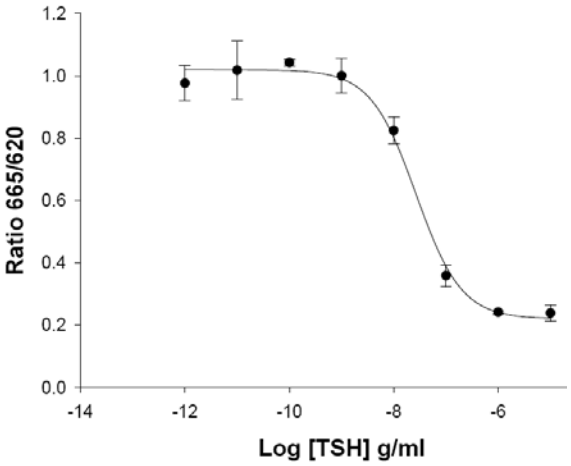

Fig.2. TSHR dose response in cAMP assay. Cells were treated with **TSH** concentrations ranging from 0 to 30 µgr/ml, n=5. The EC50 for **TSH** was 2.671x10^{-8} gr/ml. The cAMP assay was validated with a Z´= 0.76+/- 0.02 in a microplate reader.

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