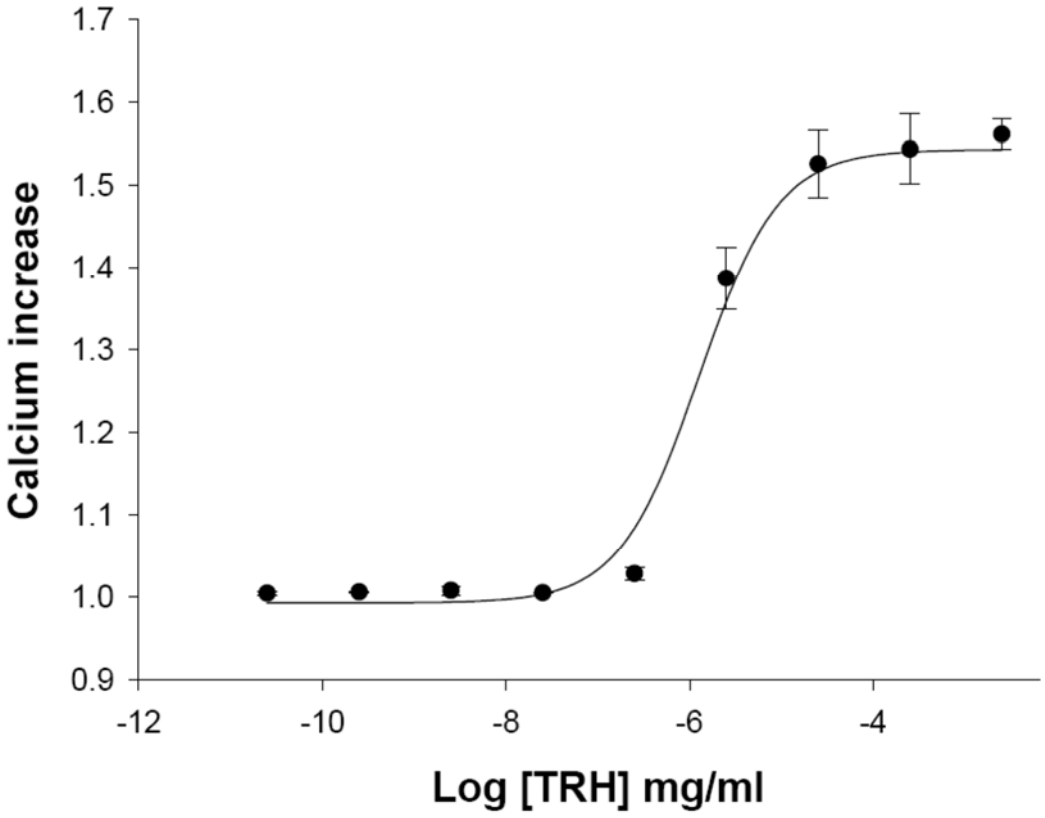
REF: P30423

HiTSeeker CELL LINES (LABEL-FREE GPCRS)

- THYROTROPIN-RELEASING HORMONE RECEPTOR (TRH1) CELL LINE -



Product name: TRH1 (TRHR) /U2OS cell line

Ec_{50} TRH: $1.26x10^{-6}$ mg/ml

Z´: 0.89+/- 0.02

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- THYROTROPIN-RELEASING HORMONE RECEPTOR (TRH1) CELL LINE -

Product Name:	TRH1/U2OS
Official Full Name:	Thyrotropin-releasing hormone receptor
DNA Accession Number:	GenBank: AY493373
Host Cell:	U2OS
Format:	2 cryopreserved vials
Resistance:	Puromycin
Size:	*P30402*: 2 vials of 3 x 10^6 proliferative cells *P30402-DA*: 1 vial of 2.5x10^6 division-arrested cells
Storage:	Liquid Nitrogen

Assay Briefly description

Each vial of HiTSeeker TRH1 contains U2OS cells stably expressing human Thyrotropin-releasing hormone receptor (TRH1) with no tag.

HiTSeeker TRH1 cell line has been designed to assay compounds or analyze their capability to modulate Thyrotropin-releasing hormone receptor. When the agonist binds to TRH1 a G protein is activated, which in turn, triggers a cellular response mediated by second messengers (Calcium).

This cell line has been validated measuring calcium increase in the cytosol. The high reproducibility of this assay allows monitoring TRH1 activation process in High Throughput Screening.

About TRH1

The thyrotropin-releasing hormone receptor (TRHR) is a G protein-coupled receptor which binds the tripeptide thyrotropin releasing hormone.

Thyrotropin-releasing hormone (**TRH**), is a, tripeptidal hormone, produced by the hypothalamus, that stimulates the release of TSH (thyroid-stimulating hormone) and prolactin from the anterior pituitary.
TRH receptor can be a good target to test thyroid disorders.

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Assay Characterization

Our expression plasmid contains the coding sequence of human TRH1 protein. Our plasmid was transfected in U2OS cells. Resistant clones were obtained by limit dilution and receptor gene expression was tested by RT-PCR using GAPDH as internal control (Fig.1).

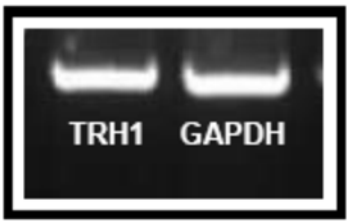

Fig.1.TRH1 and GAPDH housekeeping gene RT-PCR.

Cells were incubated with Fura2-AM and treated with increasing TRH concentrations.

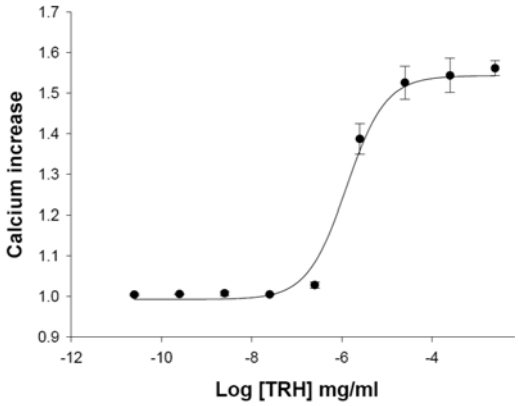

Fig.2. TRH1 dose response in calcium assay. Cells were treated with **TRH** concentrations ranging from 0 to 2.5 mg/ml, n=6. The EC50 for **TRH** was **~1.26x10^{-6} mg/ml**. The calcium assay was validated with a Z´= 0.89+/- 0.02 for High Content Screening.

Validation of TRH1 *cell line*

Calcium assay (Ec50 = 1.26 x 10^{-6}mg/ml)

A typical fluorescent calcium assay was performed using Fura-2/AM ratiometric. Calcium increase inside the cell was measured using the ratio of the fluorescence from Fura2 bound and not bound to the ion. Image acquisition was performed using a "BD Pathway 855" High-Content Bioimager from BD Biosciences.

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