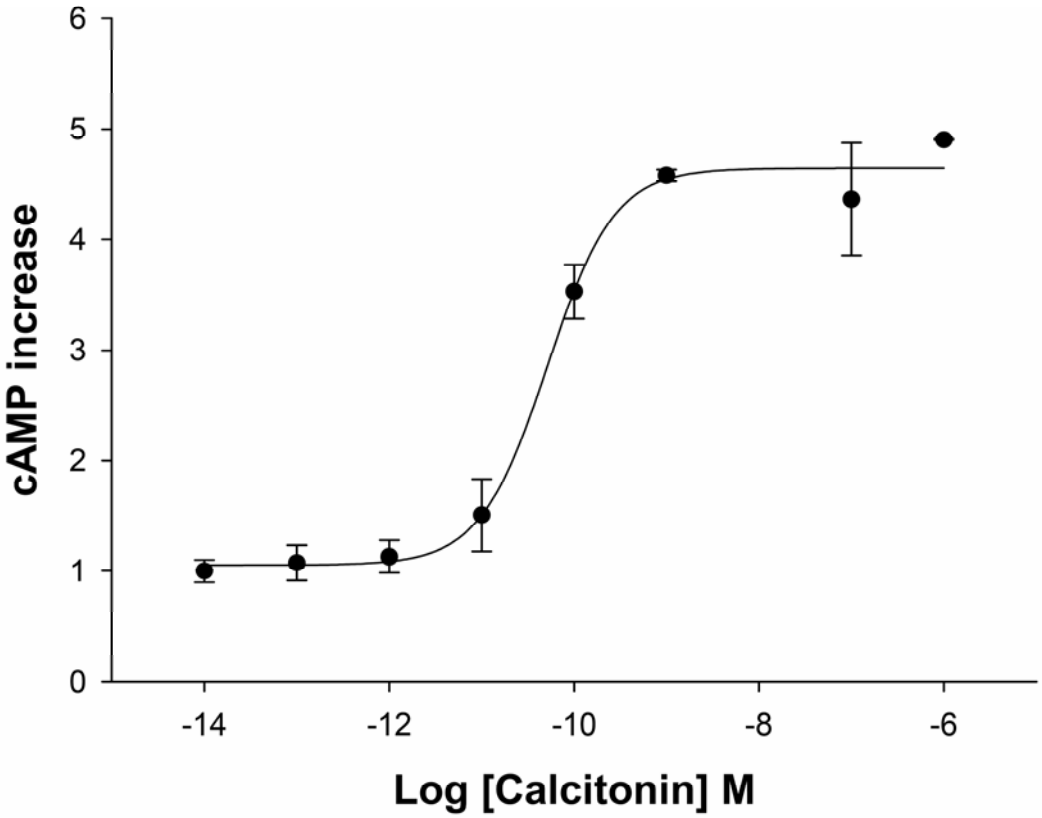
REF: P30136

HiTSeeker CELL LINES (LABEL-FREE GPCRS)

- HUMAN CALCITONIN RECEPTOR CELL LINE -



Product name: CALCR / HEK293 cell line

Ec_{50} Calcitonin (Calcium Assay): 8 x 10^{-8} M

Z´: 0.90+/- 0.01

Ec_{50} Calcitonin (cAMP Assay): 5.12 x 10^{-11} M

Z´: 0.62+/- 0.02

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HiTSeeker CELL LINES (LABEL-FREE GPCRS)

HUMAN CALCITONIN RECEPTOR CELL LINE

Product Name:	HiTSeeker CALCR cell line
Receptor Official Full Name:	Human Calcitonin Receptor
DNA Accesion Number:	GenBank AY430048
Host Cell:	HEK293 cell line
Resistance:	G418 (Geneticin)
Format:	Cryopreserved vials
Quantity:	*P30136*: 2 vials of 3 x 10^6 proliferative cells
	P30136-DA: 1 vial of 2.5x10^6 division-arrested cells
Storage:	Liquid Nitrogen

Assay Briefly description

Each vial ofr HiTSeeker CALCR contains HEK293 cells stably expressing human Calcitonin Receptor (CALCR) with no tag.

Innoprot CALCR cell line has been designed to assay compounds or analyze their capability to modulate Calcitonin receptor. When Calcitonin binds to the CALCR a G protein is activated, which in turn, triggers a cellular response mediated by second messengers (Calcium and cAMP).

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

About CALCR Receptor

This gene encodes a high affinity receptor for the peptide hormone Calcitonin and belongs to a subfamily of seven transmembrane-spanning G protein coupled receptors. The protein is involved in maintaining calcium homeostasis and in regulating osteoclast-mediated bone resorption.

Polymorphisms in this gene have been associated with variations in bone mineral density and onset of osteoporosis. Alternate splicing results in multiple transcript variants.

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Assay characterization

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

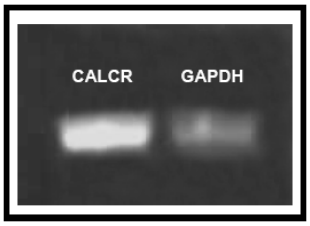

Fig.1. CALCR and GAPDH housekeeping gene RT-PCR.

Validation of CALCR cell line

Calcium assay (Ec50 = $8x10^{-8}$ M)

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.

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

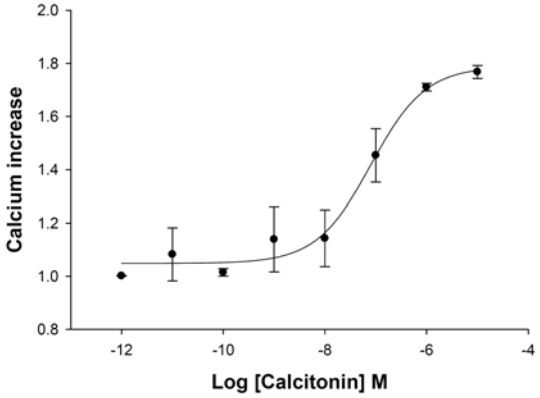

Fig.2. Calcitonin dose response in calcium assay. Cells were treated with Calcitonin concentrations ranging from 0 to 10 µM by quadruplicate. The EC50 for Calcitonin was ~ $8x10^{-8}$ M. The calcium assay was validated with a Z´= 0.9 for High Content Screening.

cAMP production assay (EC_{50} = $5.12x10^{-11}$ M)

cAMP production was assessed using the cAMP dynamic 2 kit (Cisbio). Fluorescence detection was recorded in a Multi-Mode Microplate Reader Synergy 2 from Biotek.

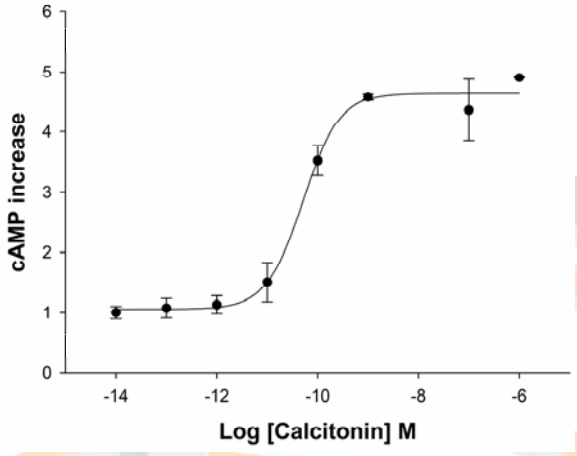

Fig.4. Calcitonin dose response curve in cAMP assay. Cells were treated with Calcitonin concentrations ranging from 0 to 1 µM by quadruplicate. The Ec50 for the Calcitonin was ~ $5.12x10^{-11}$ M. The cAMP assay was validated with an average of Z´= 0.62 for High Content Screening.

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