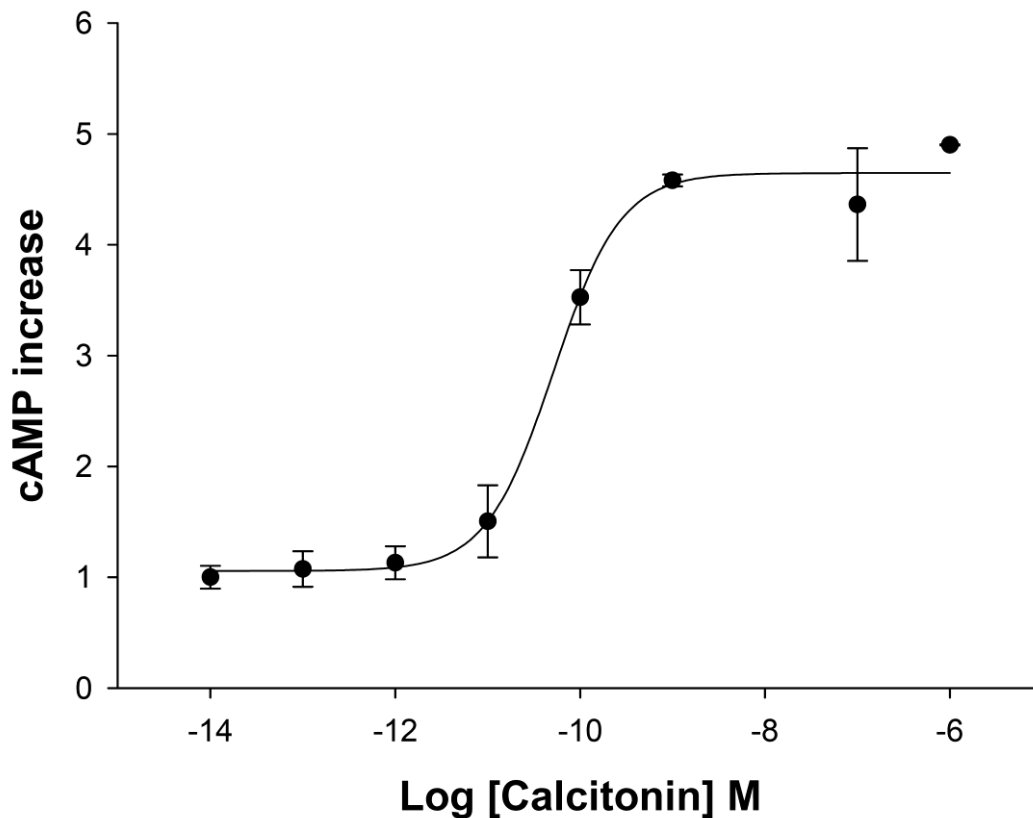


## HiTSeeker CELL LINES (LABEL-FREE GPCRS)

- HUMAN CALCITONIN RECEPTOR CELL LINE -



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**Product name:** CALCR / HEK293 cell line

**Ec<sub>50</sub> Calcitonin (Calcium Assay):**  $8 \times 10^{-8}$  M

**Z':** 0.90+/- 0.01

**Ec<sub>50</sub> Calcitonin (cAMP Assay):**  $5.12 \times 10^{-11}$  M

**Z':** 0.62+/- 0.02

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## HiTSeeker CELL LINES (LABEL-FREE GPCRS)

### HUMAN CALCITONIN RECEPTOR CELL LINE

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

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#### Assay Briefly description

Each vial of 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.

## 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).

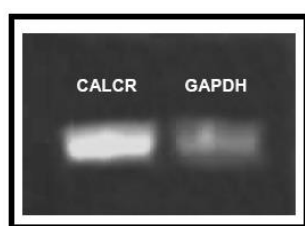


Fig.1. CALCR and GAPDH housekeeping gene RT-PCR.

## Validation of CALCR cell line

### Calcium assay ( $EC_{50} = 8 \times 10^{-8} \text{ M}$ )

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.

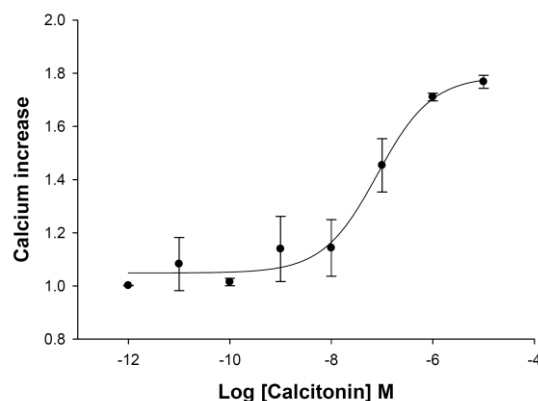


Fig.2. Calcitonin dose response in calcium assay. Cells were treated with Calcitonin concentrations ranging from 0 to 10  $\mu\text{M}$  by quadruplicate. The  $EC_{50}$  for Calcitonin was  $\sim 8 \times 10^{-8} \text{ M}$ . The calcium assay was validated with a  $Z' = 0.9$  for High Content Screening.

### cAMP production assay ( $EC_{50} = 5.12 \times 10^{-11} \text{ M}$ )

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.

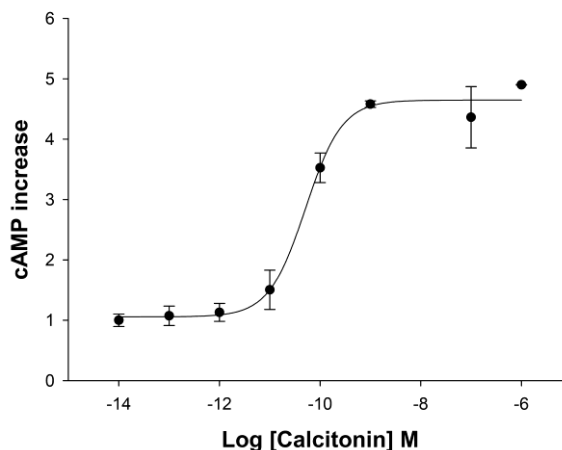


Fig.4. Calcitonin dose response curve in cAMP assay. Cells were treated with Calcitonin concentrations ranging from 0 to 1  $\mu\text{M}$  by quadruplicate. The  $EC_{50}$  for the Calcitonin was  $\sim 5.12 \times 10^{-11} \text{ M}$ . The cAMP assay was validated with an average of  $Z' = 0.62$  for High Content Screening.