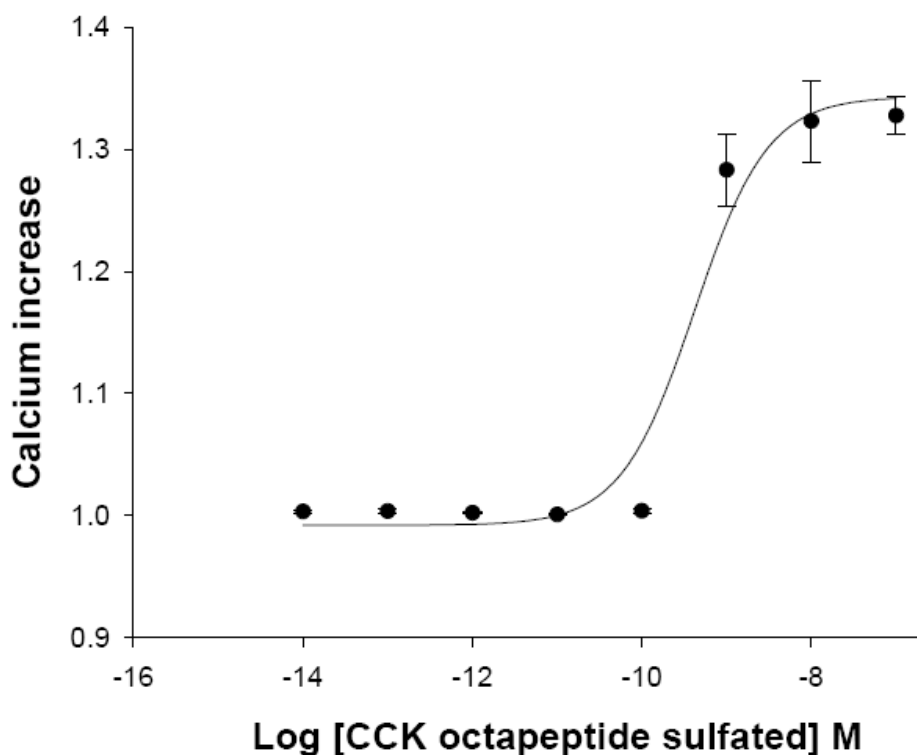


## HiTSeeker CELL LINES (LABEL-FREE GPCRS)

- CHOLECYSTOKININ A RECEPTOR (CCK<sub>1</sub>) CELL LINE -



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**Product name:** CCK<sub>1</sub> (CCKAR) /U2OS cell line

**Ec<sub>50</sub> CCK Octapeptide, sulfated:** 4.17x 10<sup>-10</sup> M

**Z':** 0.84 +/- 0.02

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## - CHOLECYSTOKININ A RECEPTOR (CCK<sub>1</sub>) U2OS CELL LINE -

<b>Product Name:</b>	CCK <sub>1</sub> (CCKAR)/U2OS
<b>Official Full Name:</b>	Cholecystokinin A receptor
<b>DNA Accession Number:</b>	GenBank: AY322549
<b>Host Cell:</b>	U2OS
<b>Format:</b>	2 cryopreserved vials
<b>Resistance:</b>	G418
<b>Size:</b>	> 3 x 10 <sup>6</sup> cells / vial
<b>Storage:</b>	Liquid Nitrogen

### **Assay Briefly description**

Each vial of HiTSeeker CCK<sub>1</sub> contains U2OS cells stably expressing human Cholecystokinin A receptor (CCK<sub>1</sub>) with no tag.

Innoprot HiTSeeker CCK<sub>1</sub> cell line has been designed to assay compounds or analyze their capability to modulate Cholecystokinin A receptor. When the agonist binds to CCK<sub>1</sub>, 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 CCK<sub>1</sub> activation process in High Throughput Screening.

### **About CCK<sub>1</sub>**

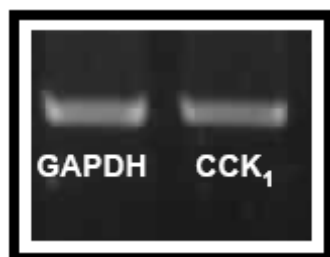
CCK receptors family is composed of two GPCRs known as CCK<sub>1</sub> and CCK<sub>2</sub> receptors. Both receptors bind Cholecystokinin (CCK) that is a main gastrointestinal and neuronal peptide hormone, involved in stimulating gallbladder contraction, pancreatic secretion, gastrointestinal motility and satiety.

The CCK<sub>1</sub> receptor has been implicated in several gastrointestinal diseases and it has a relevant role in modulating food consumption, which is very interesting field to study for a treatment for obesity.

CCK<sub>1</sub> receptor has been implicated too in small-cell lung and/or pancreatic cancer types.

## Assay Characterization

Our expression plasmid contains the coding sequence of human CCK<sub>1</sub> 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).



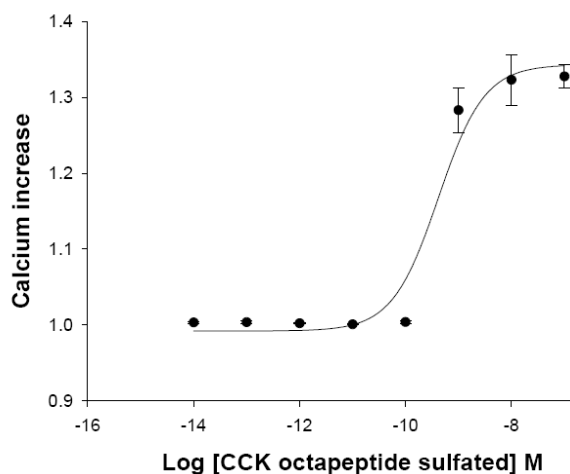
**Fig.1. GAPDH housekeeping gene and CCK<sub>1</sub> RT-PCR.**

## Validation of CCK<sub>1</sub> cell line

### Calcium assay (EC<sub>50</sub> = 4.17 x 10<sup>-10</sup> 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 CCK Octapeptide, sulfated concentrations.



**Fig.2. CCK<sub>1</sub> dose response in calcium assay.**

Cells were treated with CCK Octapeptide, sulfated concentrations ranging from 0 to 100 nM, n=5. The EC<sub>50</sub> for CCK Octapeptide, sulfated was ~ 4.17x10<sup>-10</sup>M. The calcium assay was validated with a Z' = 0.84 +/- 0.02 for High Content Screening.