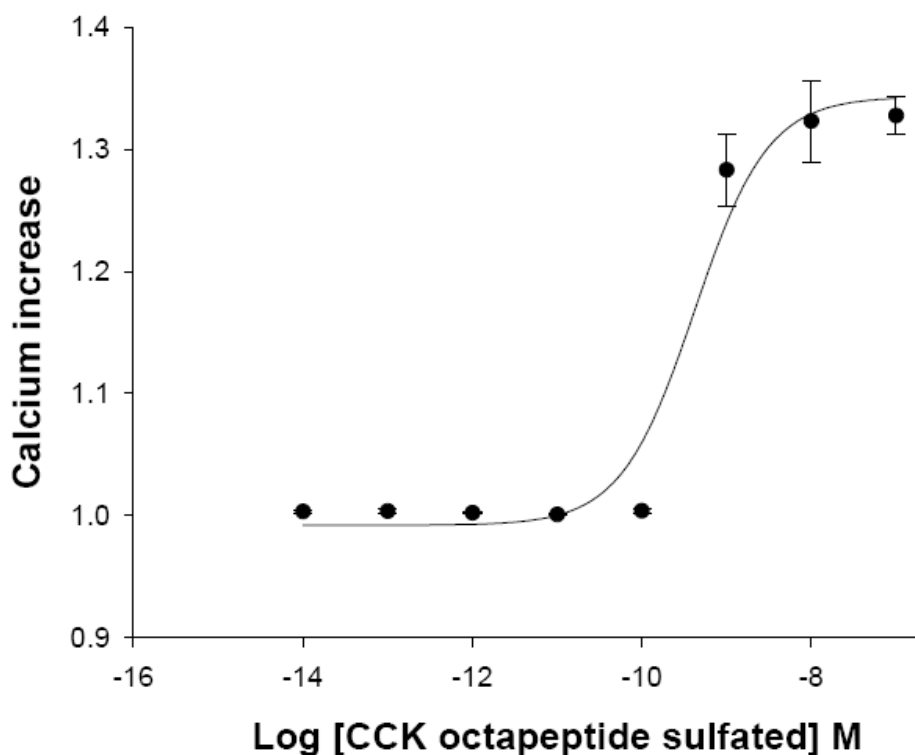


HiTSeeker CELL LINES (LABEL-FREE GPCRS)

- CHOLECYSTOKININ A RECEPTOR (CCK₁) CELL LINE -



Product name: CCK₁ (CCKAR) /U2OS cell line

Ec₅₀ CCK Octapeptide, sulfated: 4.17x 10⁻¹⁰ M

Z': 0.84 +/- 0.02

- CHOLECYSTOKININ A RECEPTOR (CCK₁) U2OS CELL LINE -

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

Assay Briefly description

Each vial of HiTSeeker CCK₁ contains U2OS cells stably expressing human Cholecystokinin A receptor (CCK₁) with no tag.

Innoprot HiTSeeker CCK₁ cell line has been designed to assay compounds or analyze their capability to modulate Cholecystokinin A receptor. When the agonist binds to CCK₁, 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 CCK₁ activation process in High Throughput Screening.

About CCK₁

CCK receptors family is composed of two GPCRs known as CCK₁ and CCK₂ 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 CCK₁ 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.

CCK₁ 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 CCK₁ 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).

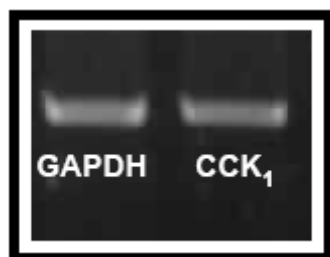


Fig.1. GAPDH housekeeping gene and CCK₁ RT-PCR.

Validation of CCK₁ cell line

Calcium assay (EC₅₀ = 4.17 x 10⁻¹⁰ 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.

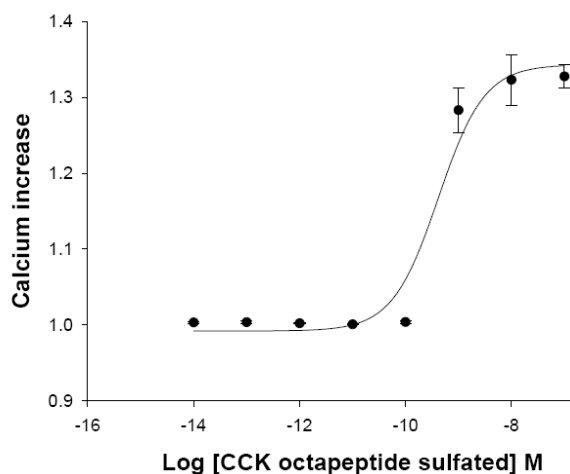


Fig.2. CCK₁ dose response in calcium assay.

Cells were treated with CCK Octapeptide, sulfated concentrations ranging from 0 to 100 nM, n=5. The EC₅₀ for CCK Octapeptide, sulfated was ~ 4.17x10⁻¹⁰M. The calcium assay was validated with a Z' = 0.84 +/- 0.02 for High Content Screening.