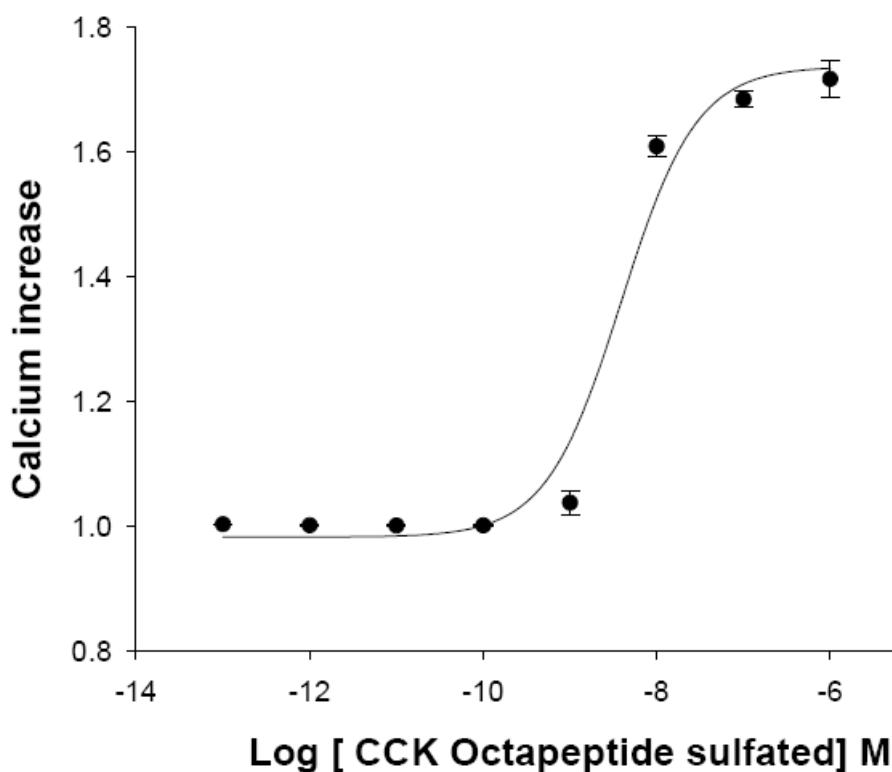


HiTSeeker CELL LINES (LABEL-FREE GPCRS)
- CHOLECYSTOKININ B RECEPTOR (CCK₂) CELL LINE -



Product name: CCK₂ (CCKBR) /U2OS cell line

Ec₅₀ CCK Octapeptide, sulfated: 3.9 x 10⁻⁹ M

Z': 0.87+/- 0.02

- CHOLECYSTOKININ B RECEPTOR (CCK₂) U2OS CELL LINE -

Product Name:	CCK ₂ (CCKBR)/U2OS
Official Full Name:	Cholecystokinin A receptor
DNA Accession Number:	GenBank: AY322551
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 CCK₂/U2OS contains U2OS cells stably expressing human Cholecystokinin B receptor (CCK₂) with no tag.

Innoprot HiTSeeker CCK₂ cell line has been designed to assay compounds or analyze their capability to modulate Cholecystokinin B receptor. When the agonist binds to CCK₂ 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 CCK₂ activation process in High Throughput Screening.

About CCK₂

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 CCK₁₂ receptor has a high expression in several tumour types including medullary thyroid carcinoma (MTC), neuroendocrine tumours, small cell lung cancer, and colorectal cancers.

Assay Characterization

Our expression plasmid contains the coding sequence of human CCK₂ 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).

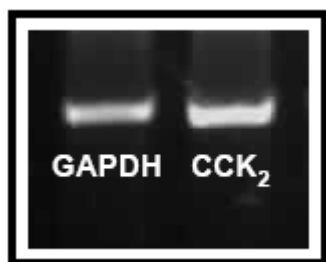


Fig.1. GAPDH housekeeping gene and CCK₂, RT-PCR.

Validation of CCK₂ cell line

Calcium assay (EC₅₀ = 3.9 × 10⁻⁹M)

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.

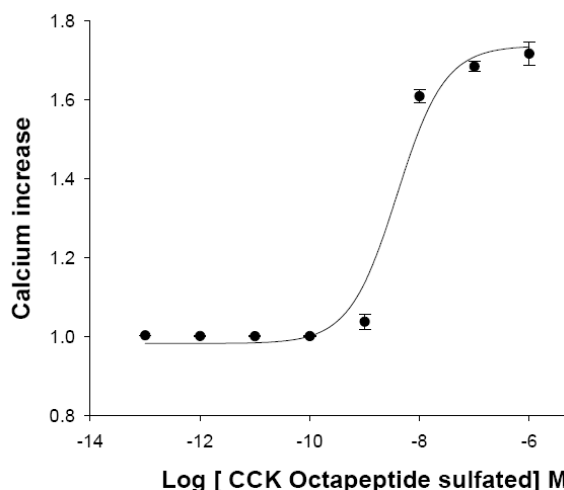


Fig.2. CCK₂ dose response in calcium assay. Cells were treated with CCK Octapeptide, sulfated concentrations ranging from 0 to 1 μM, n=5. The EC₅₀ for **CCK Octapeptide, sulfated** was ~ **3.9 × 10⁻⁹ M**. The calcium assay was validated with a Z' = 0.87 ± 0.02 for High Content Screening.