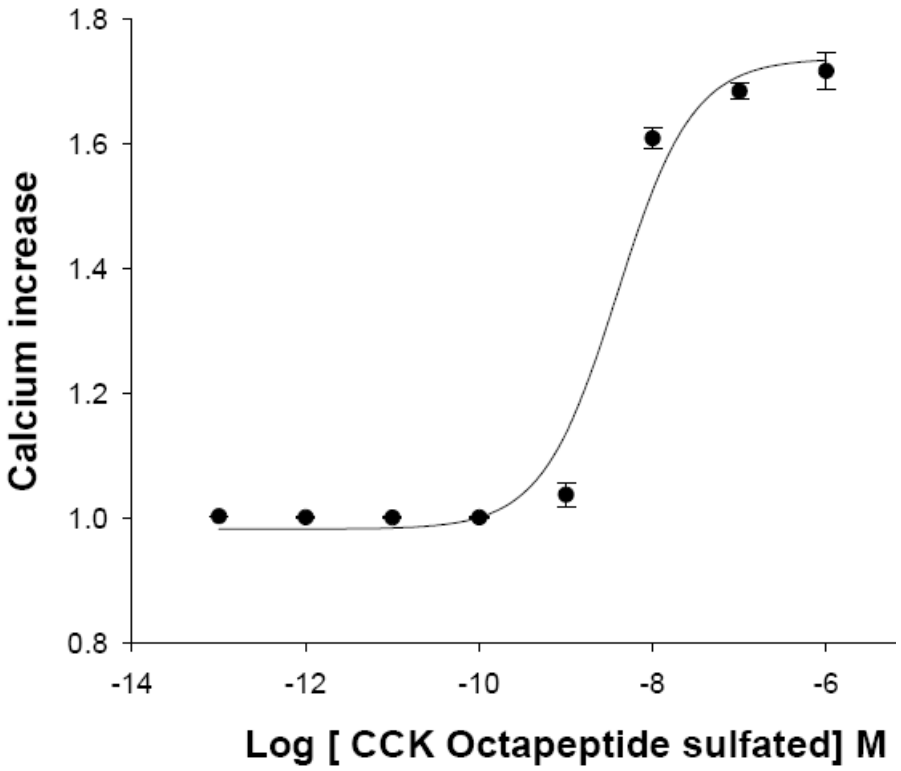
REF: P30179

HiTSeeker CELL LINES (LABEL-FREE GPCRS)

- CHOLECYSTOKININ B RECEPTOR (CCK_2) CELL LINE -



Product name: CCK_2 (CCKBR) /U2OS cell line

Ec_{50} CCK Octapeptide, sulfated: 3.9 x 10^{-9} M

Z´: 0.87+/- 0.02

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REF: P30179

- CHOLECYSTOKININ B RECEPTOR (CCK_2) 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 CCK_2/U2OS contains U2OS cells stably expressing human Cholecystokinin B receptor (CCK_2) with no tag.

Innoprot HiTSeeker CCK_2 cell line has been designed to assay compounds or analyze their capability to modulate Cholecystokinin B receptor. When the agonist binds to CCK_2 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_2 activation process in High Throughput Screening.

About CCK_2

CCK receptors family is composed of two GPCRs known as CCK_1 and CCK_2 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_{12} receptor has a high expression in several tumour types including medullary thyroid carcinoma (MTC), neuroendocrine tumours, small cell lung cancer, and colorectal cancers.

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Assay Characterization

Our expression plasmid contains the coding sequence of human CCK_2 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).

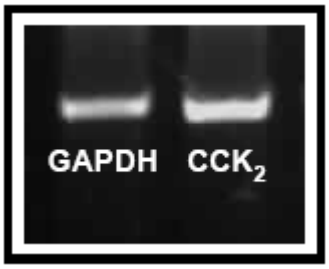

Fig.1. GAPDH housekeeping gene and $CCK2_1$ RT-PCR.

Validation of CCK_2 cell line

Calcium assay (Ec50 = 3.9 x 10^{-9}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.

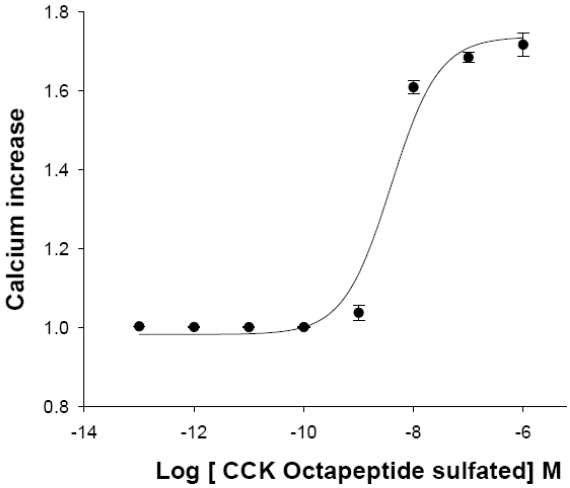

Fig.2. CCK_2 **dose response in calcium assay.** Cells were treated with CCK Octapeptide, sulfated concentrations ranging from 0 to 1 μM, n=5. The EC50 for **CCK Octapeptide, sulfated** was ~ **$3.9x10^{-9}$M**. The calcium assay was validated with a Z´= 0.87+/- 0.02 for High Content Screening.

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