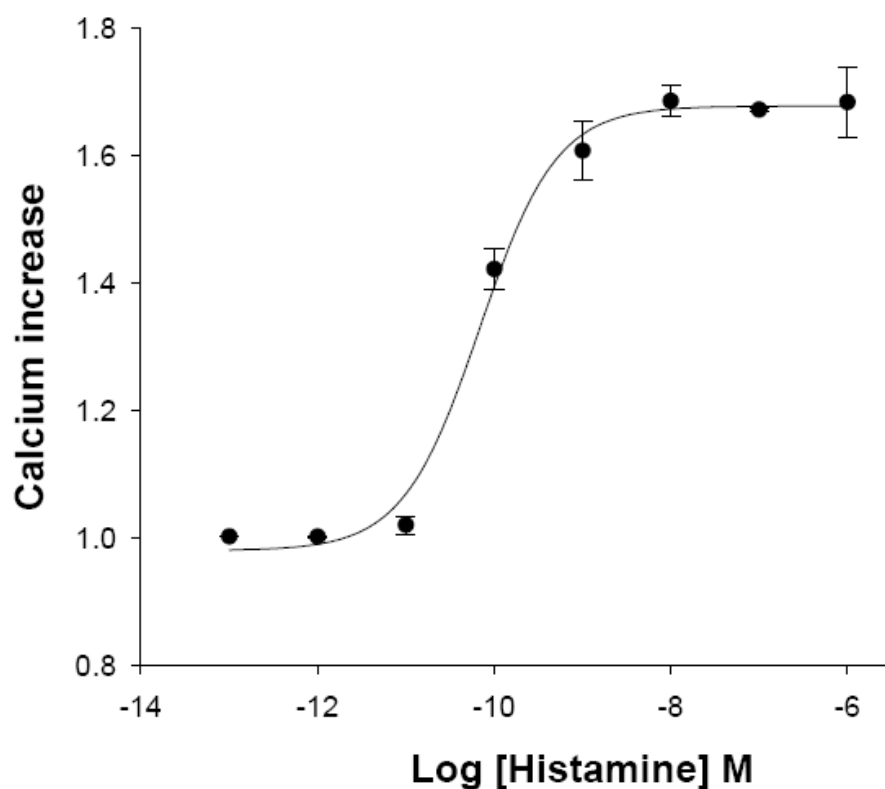


## **HiTSeeker CELL LINES (LABEL-FREE GPCRS)**

### **- HISTAMINE RECEPTOR 1 (HRH1) CELL LINE -**





**Product name:** HRH1 /U2OS cell line

**Ec<sub>50</sub> Histamine dihydrochloride:**  $2.74 \times 10^{-11}$  M

**Z':** 0.76 $\pm$  0.02

## - HISTAMINE RECEPTOR 1 (HRH1) U2OS CELL LINE -

<b>Product Name:</b>	HRH1/U2OS
<b>Official Full Name:</b>	Histamine H1 receptor
<b>DNA Accession Number:</b>	GenBank: AY136743 / D28481
<b>Host Cell:</b>	U2OS
<b>Format:</b>	2 cryopreserved vials
<b>Resistance:</b>	G418
<b>References:</b>	
	 <b>P30183:</b> 2 vials of $3 \times 10^6$ proliferative cells
	 <b>P30183-DA:</b> 1 vial of $2 \times 10^6$ division-arrested cells
<b>Storage:</b>	Liquid Nitrogen

### **Assay Briefly description**

Each vial of HiTSeeker HRH1 contains U2OS cells stably expressing human Histamine H1 receptor (HRH1) with no tag.

Innoprot's HiTSeeker HRH1 cell line has been designed to assay compounds or analyze their capability to modulate Histamine H1 receptor. When the agonist binds to HRH1 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 HRH1 activation process in High Throughput Screening.

### **About HRH1**

The histamine receptors are a class of G protein-coupled receptors with histamine as their endogenous ligand.

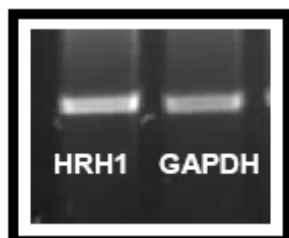
There are four known histamine receptors: HRH1, HRH2, HRH3 and HRH4.

Histamine H1 receptor is highly distributed in several tissues such as central nervous system, cardiovascular system, smooth muscles, or lymphocytes.

HRH1 is a target for the treatment of allergic diseases.

## **Assay Characterization**

Our expression plasmid contains the coding sequence of human HRH1 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).



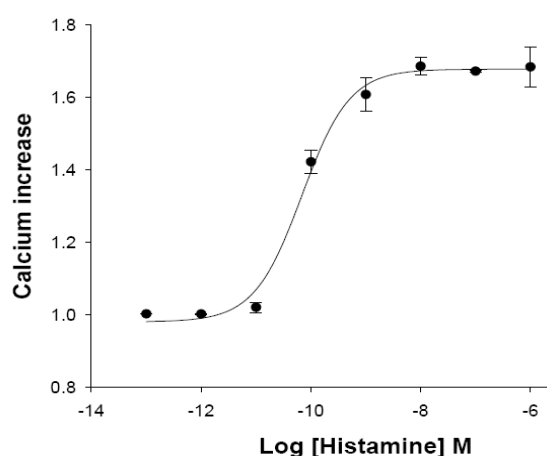
**Fig.1. HRH1 and GAPDH housekeeping gene RT-PCR.**

## **Validation of HRH1 cell line**

### **Calcium assay ( $EC_{50} = 2.74 \times 10^{-11}M$ )**

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 Histamine **dihydrochloride** concentrations.



**Fig.2. HRH1 dose response in calcium assay.**

Cells were treated with Histamine **dihydrochloride** concentrations ranging from 0 to 1  $\mu M$ ,  $n=5$ . The  $EC_{50}$  for Histamine **dihydrochloride** was  $2.74 \times 10^{-11}M$ . The calcium assay was validated with a  $Z' = 0.76 \pm 0.02$  for High Throughput Screening.