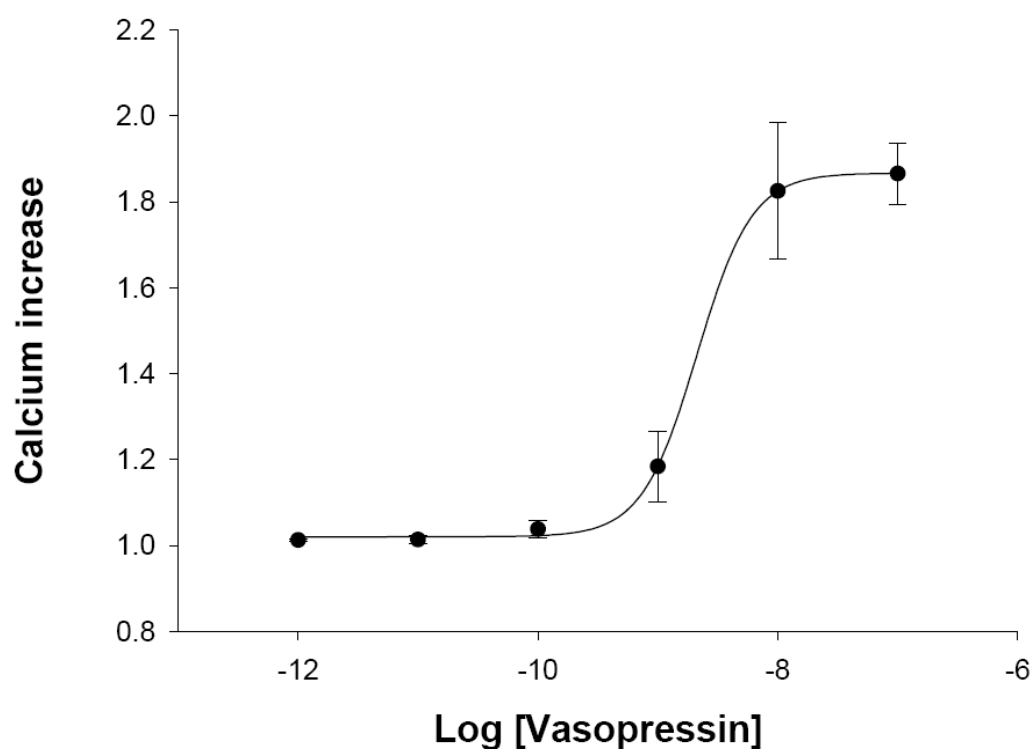


## HiTSeeker CELL LINES (LABEL-FREE GPCRS)

- ARGININE VASOPRESSIN RECEPTOR 1A HEK293 CELL LINE -



**Product name:** AVPR1A / HEK293 cell line

**Ec<sub>50</sub> Vasopressin:**  $2.1 \times 10^{-9}$  M

**Z':** 0.74 $\pm$  0.02

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

### **ARGININE VASOPRESSIN RECEPTOR 1A CELL LINE**

<b>Product Name:</b>	AVPR1A /HEK293
<b>Official Full Name:</b>	Arginine Vasopressin receptor 1A
<b>DNA Accession Number:</b>	GenBank: NM_000706
<b>Host Cell:</b>	HEK293
<b>Format:</b>	2 cryopreserved vials
<b>Resistance:</b>	Puromycin
<b>Product References:</b>	<i>P30103</i> : 2 vials of $3 \times 10^6$ proliferative cells <i>P30103-DA</i> : 1 vial of $2.5 \times 10^6$ division-arrested cells
<b>Storage:</b>	Liquid Nitrogen

#### **Assay Briefly description**

AVPR1A/HEK293 contains HEK293 cells stably expressing human Arginine Vasopressin receptor 1A with no tag.

InnoproT AVPR1A cell line has been designed to assay compounds or analyze their capability to modulate Arginine Vasopressin receptor 1A. When the agonist binds to AVPR1A a G protein is activated, which in turn, triggers a cellular response mediated by second messengers (Ca).

This cell line has been validated measuring Calcium increase in the cytosol. The high reproducibility of this assay allows monitoring AVPR1A activation process in High Throughput Screening.

#### **About AVPR1A1**

The activity of Arginine Vasopressin receptor 1A (AVPR1A) is mediated by G proteins which stimulate a phosphatidylinositol-calcium second messenger system. Arginine Vasopressin 1A receptor belongs to the same subfamily of G-protein coupled receptors of AVPR1B, V2R and Oxytocin receptors.

The receptor mediates cell contraction and proliferation, platelet aggregation, release of coagulation factor and glycogenolysis. V1A is coupled to Gq/11 proteins and is found at high density on smooth muscle cells. It is involved in regulation of blood pressure through arterial vasoconstriction and has been found to stimulate VEGF secretion. AVPR1A is widely expressed in the brain and it has been implicated in social behaviours, including affiliation and attachment and related to diseases as autism.

## **Assay Characterization**

Our expression plasmid contains the coding sequence of human AVPR1A receptor protein. Our plasmid was transfected in HEK293 cells. Resistant clones were obtained by limit dilution and receptor gene expression was tested by RT-PCR using GAPDH as internal control (Fig.1).



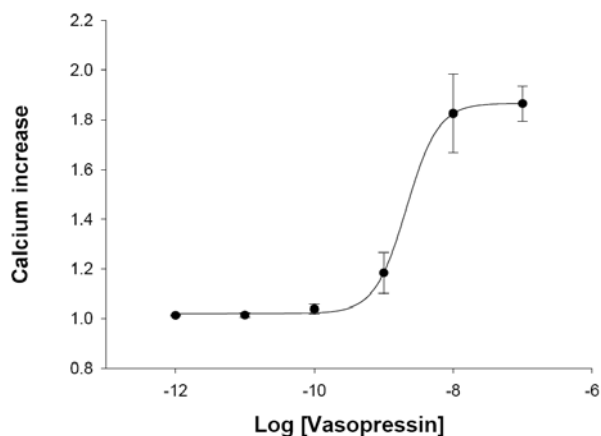
**Fig.1. AVPR1A and GAPDH housekeeping gene RT-PCR.**

## **Validation of AVPR1A cell line**

### **Calcium assay ( $EC_{50} = 2.1 \times 10^{-9} M$ )**

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 Vasopressin concentrations.



**Fig.2. Vasopressin dose response curve in calcium assay.**

Cells were treated with Vasopressin. Concentrations from 0 to 100 nM were tested by quadruplicate. The  $EC_{50}$  for the Vasopressin is  $2.1 \times 10^{-9} M$ . The calcium assay was validated with a  $Z' = 0.74$  for High Content Screening.