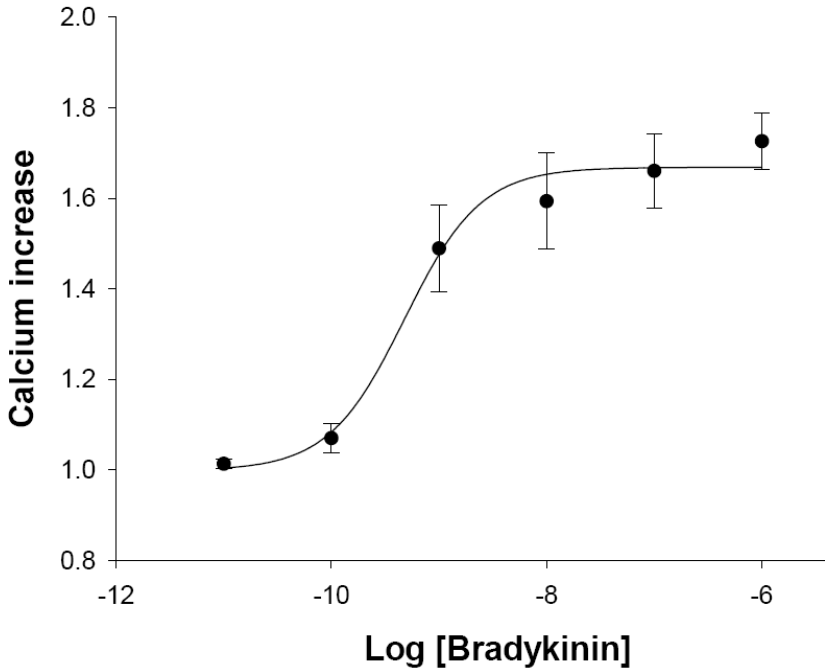
REF: P30110

HiTSeeker CELL LINES (LABEL-FREE GPCRS)

-BRADYKININ RECEPTOR B2 CELL LINE -



Product name: HiTSeeker BDKRB2 cell line

Host Cell Lines: U2OS & HEK293

Ec_{50} Bradykinin: 4.77 x 10^{-10} M (HEK293)

2.75x 10^{-9} M (U2OS)

Z´: 0.70+/- 0.02

INNOVATIVE TECHNOLOGIES IN BIOLOGICAL SYSTEMS, S.L.
Parque Tecnológico Bizkaia, Edifício 502, 1ª Planta | 48160 | Derio | Bizkaia
Tel.: +34 944005355 | Fax: +34 946579925
innoprot@innoprot.com | www.innoprot.com

REF: P30110

- BRADYKININ RECEPTOR B2 HEK293 CELL LINE -

Product Name:	HiTSeeker BDKRB2
Official Full Name:	Bradykinin receptor 2
DNA Accesion Number:	GenBank: AY275465
Host Cell:	HEK293 & U2OS
Format:	2 cryopreserved vials
Resistance:	Puromycin
References:	*P30110U*: 2 vials of 3 x 10^6 proliferative cells (U2OS)
	P30110U-DA: 1 vial of 2.5x10^6 division-arrested cells (U2OS)
	P30110H: 2 vials of 3 x 10^6 proliferative cells (HEK293)
	P30110H-DA: 1 vial of 2.5x10^6 division-arrested cells (HEK293)
Storage:	Liquid Nitrogen

Assay Briefly description

HiTSeeker BDKRB2 contains HEK293 or U2OS cells stably expressing human Bradykinin receptor 2 (BDKRB2) with no tag.

HiTSeeker BDKRB2 cell line has been designed to assay compounds or analyze their capability to modulate Bradykinin receptor 2. When the agonist binds to BDKRB2 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 BDKRB2 activation process in High Throughput Screening.

About BDKRB2

Bradykinin receptor B_2 is a G-protein coupled receptor for Bradykinin, encoded by the **BDKRB2** gene in humans.

The B_2 receptor is a G protein-coupled receptor that stimulates phospholipase C to increase intracellular free calcium.

The 9 amino acid Bradykinin peptide elicits many responses including vasodilation, edema, smooth muscle spasm and pain fiber stimulation.

INNOVATIVE TECHNOLOGIES IN BIOLOGICAL SYSTEMS, S.L.
Parque Tecnológico Bizkaia, Edifício 502, 1ª Planta | 48160 | Derio | Bizkaia
Tel.: +34 944005355 | Fax: +34 946579925
innoprot@innoprot.com | www.innoprot.com

Characterization (HEK293)

Our expression plasmid contains the coding sequence of human BDKRB2 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).

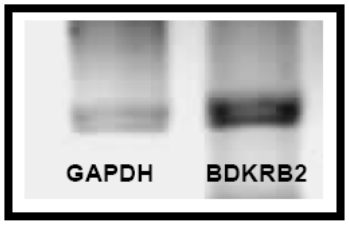


Fig.1. GAPDH housekeeping gene and BDKRB2 RT-PCR.

Validation of BDKRB2 *cell line*

Calcium assay (Ec50 = 4.77 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 Bradykinin concentrations.

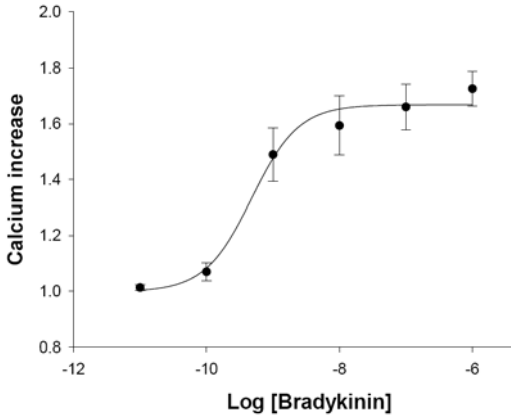


Fig.2. BDKRB2 dose response in calcium assay. Cells were treated with **Bradykinin** concentrations ranging from 0 to 1 µM, n=6. The EC50 for **Bradykinin** was ~**4.77x10^{-10}M**. The calcium assay was validated with a Z´= 0.70+/- 0.02 for High Content Screening.

INNOVATIVE TECHNOLOGIES IN BIOLOGICAL SYSTEMS, S.L.
Parque Tecnológico Bizkaia, Edifício 502, 1ª Planta | 48160 | Derio | Bizkaia
Tel.: +34 944005355 | Fax: +34 946579925
innoprot@innoprot.com | www.innoprot.com

Characterization (U2OS)

Our expression plasmid contains the coding sequence of human BK2 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).

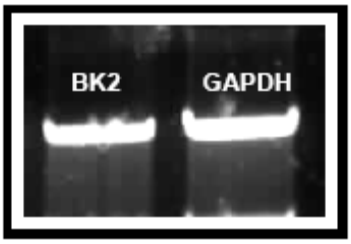


Fig.1. BK2 and GAPDH housekeeping gene RT-PCR.

Validation of BK2 cell line

Calcium assay (Ec50 = 2.75 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 Bradykinin concentrations.

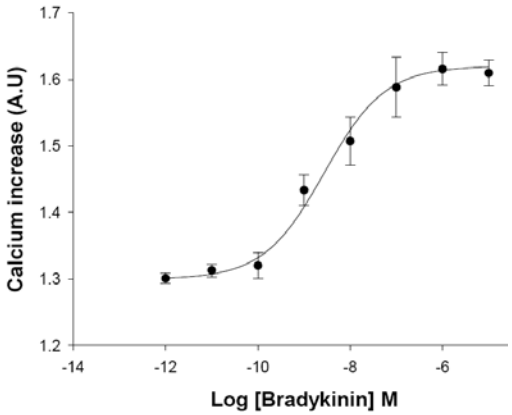


Fig.2. BK2 **dose response in calcium assay.** Cells were treated with **Bradykinin** concentrations ranging from 0 to 10 µM, n=5. The EC50 for **Bradykinin** was ~**2.75x10^{-9}M**. The calcium assay was validated with a Z´= 0.74+/- 0.02 for High Content Screening.

INNOVATIVE TECHNOLOGIES IN BIOLOGICAL SYSTEMS, S.L.
Parque Tecnológico Bizkaia, Edifício 502, 1ª Planta | 48160 | Derio | Bizkaia
Tel.: +34 944005355 | Fax: +34 946579925
innoprot@innoprot.com | www.innoprot.com