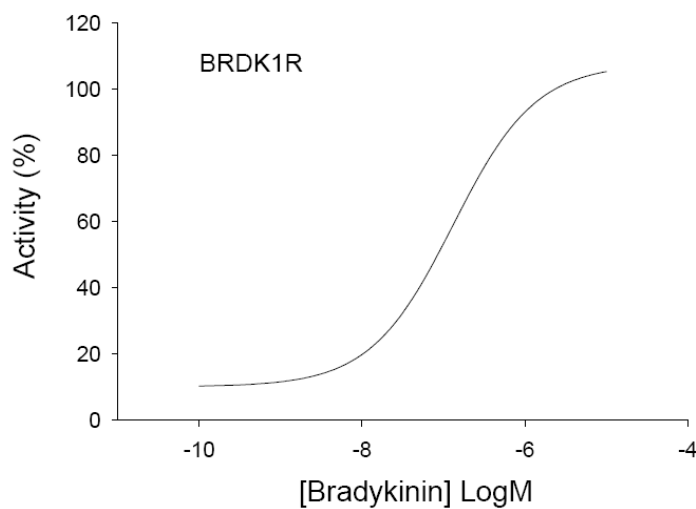
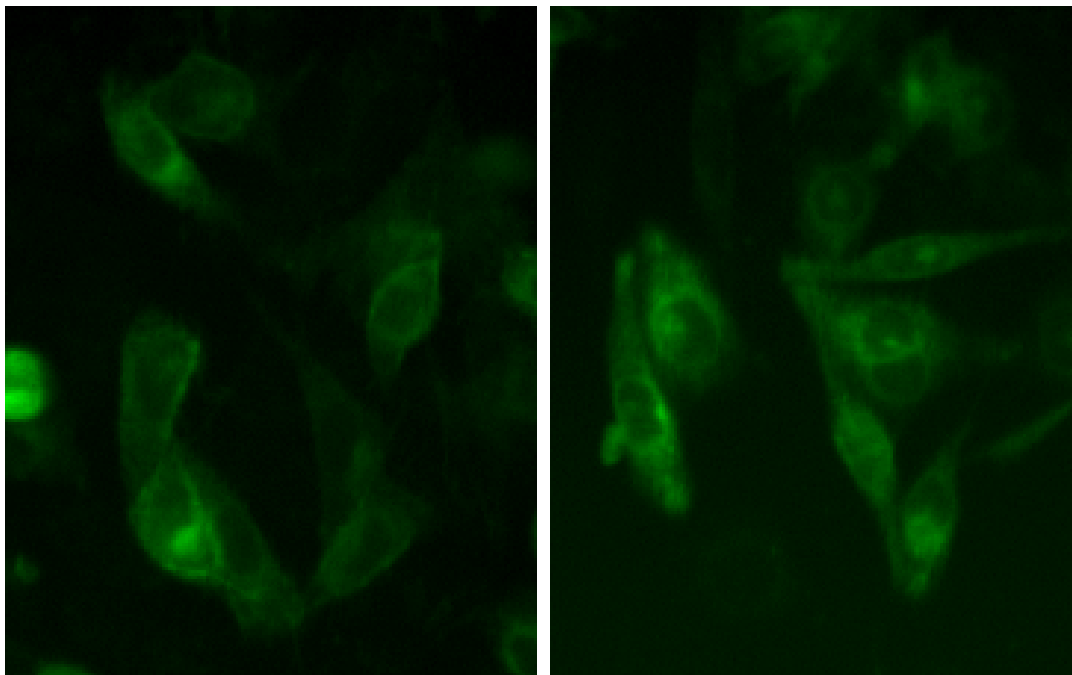


RECEPTOR INTERNALIZATION ASSAYS

- FLUORESCENT BRADYKININ β_1 RECEPTOR CELL LINE -



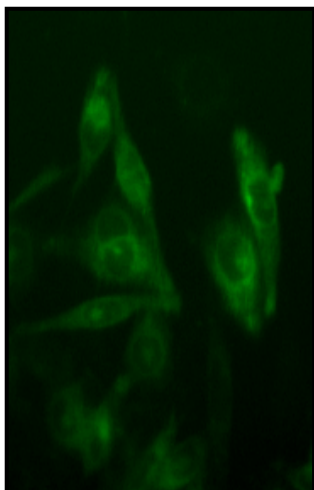
Product name: BDKR β_1 -tGFP / CHOK1 cell line

EC₅₀ Bradykinin: 1.30×10^{-7} M

Z': 0.76 \pm 0.01

RECEPTOR INTERNALIZATION ASSAYS

FLUORESCENT HUMAN BRADYKININ RECEPTOR β 1 CELL LINE



Product Name: BDKRB $_1$ tGFP/CHOK1


Receptor Official Name: Human Bradykinin receptor β 1


DNA Accesion Number: GenBank NM_000710

Host Cell: CHOK1

Format: Cryopreserved vials

References:

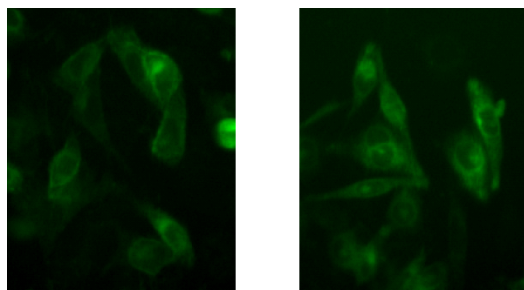
 **P30209:** 2 vials of 3×10^6 proliferative cells

 **P30209-DA:** 1 vial of 2×10^6 division-arrested cells

Storage: Liquid Nitrogen

Assay Briefly description

BDK1R-tGFP/CHO-K1 contains CHO-K1 cells stably expressing human Bradykinin receptor 1 (BDK1) tagged in the N-terminus with tGFP. Innoprot BDK1 internalization Assay kit has been designed to assay compounds or analyze stimuli for their ability to modulate Bradykinin receptor activation and the following internalization process quantifying the fluorescence distribution inside the cells.



This highly reproducible assay allows monitoring bradykinin receptor internalization in High Content Analysis and fluorescence microscope applications.

Background

Bradykinin receptor B1. BDKRB1 gene encodes a protein that is one of two Bradykinin receptors. The bradykinin receptor family is a group of G-protein coupled receptors whose principal ligand is the protein bradykinin. This gene encodes a receptor for bradykinin B1. The 9 aa bradykinin peptide elicits many responses including vasodilation, edema, smooth muscle spasm and pain fiber stimulation. This receptor associates with G proteins that stimulate a phosphatidylinositol-calcium second messenger system.

Use Restriction

This product contains a proprietary nucleic acid coding for a proprietary fluorescent protein intended to be used for research purposes only. No rights are conveyed to modify or clone the gene encoding fluorescent protein contained in this product, or to use the gene or protein other than for non-commercial research, including use for validation or screening compounds. For information on commercial licensing, contact Licensing Department, Evrogen JSC, email: license@evrogen.com

Assay Characterization

Our expression plasmid containing the coding sequence of human Bradikinin receptor 1 tagged in the N-terminal with tGFP protein. Our plasmid was transfected in CHO-K1 cells, using calcium phosphate method. Resistant clones were obtained by limit dilution, and receptor gene expression was tested by RT-PCR (Fig.1).



Fig.1. Clones BDK1 mRNA expression.

Activation and Internalization assay for BDK1R-tGFP

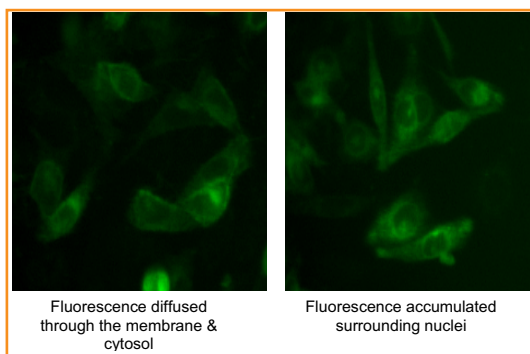
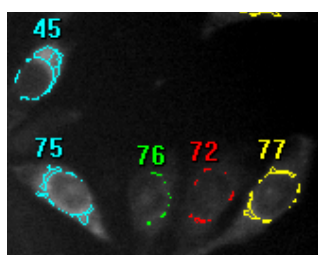


Fig.2. Internalization of BDK1-tGFP stimulated with Bradykinin. Cells were treated with 10uM Bradykinin for 5h. Activation and internalization processes were detected and analyzed using "BD Pathway 855" High-Content Bioimager from BD Biosciences.



Segmentation used in order to detect and analyze the fluorescence around the nuclei

Assay Details

CHO-K1 stably expressing human Bradykinin receptor 1 tagged in the N-terminus with tGFP were stimulated with different concentrations of Bradykinin agonist during 5 hours. After treatment an accumulation of fluorescence was observed around the nucleus. Nuclei were stained with DAPI and BDK1R redistribution was determined as the increase of fluorescence surrounding the nuclei using image analysis algorithms.

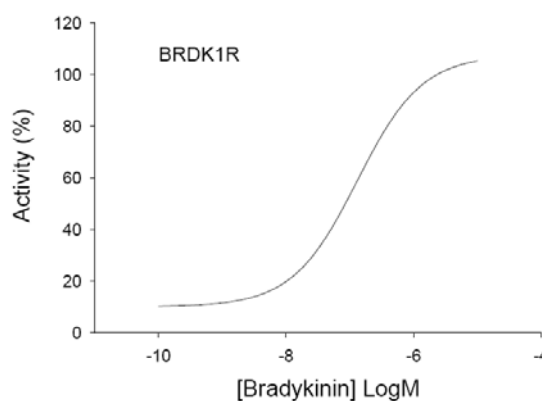


Fig.3. BDK1R-tGFP internalization in response to Bradykinin concentrations. Cells were treated with 8 log dilution series (n=8). The EC_{50} for the Bradykinin was $\sim 130nM$ after a treatment of 5h with agonist. Cells were fixed and the nuclei were stained with DAPI. % Activity was calculated relative to positive (10uM). The internalization assay was validated with an average of $Z' = 0.76 \pm 0.01$ for High Content Screening.

Quality Control

All cells are performance assayed and test negative for mycoplasma, bacteria, yeast and fungi. Cell viability, morphology and proliferative capacity are measured after recovery from cryopreservation. Innoprot guarantees stable expression for many generations and provides support for cell culture and visualization.