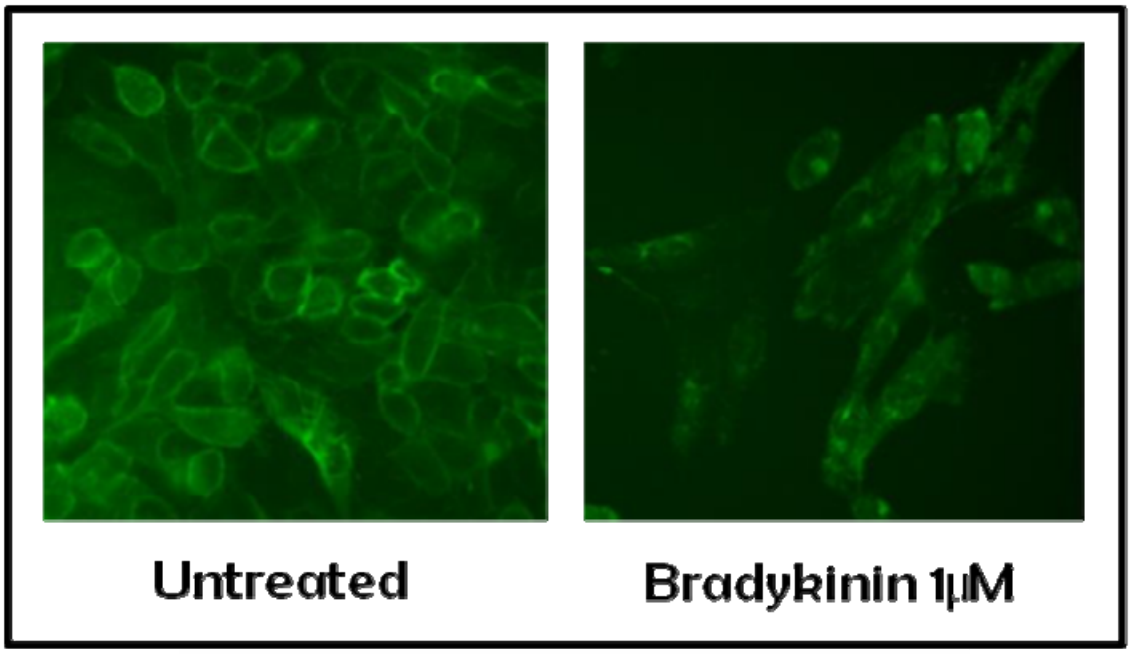
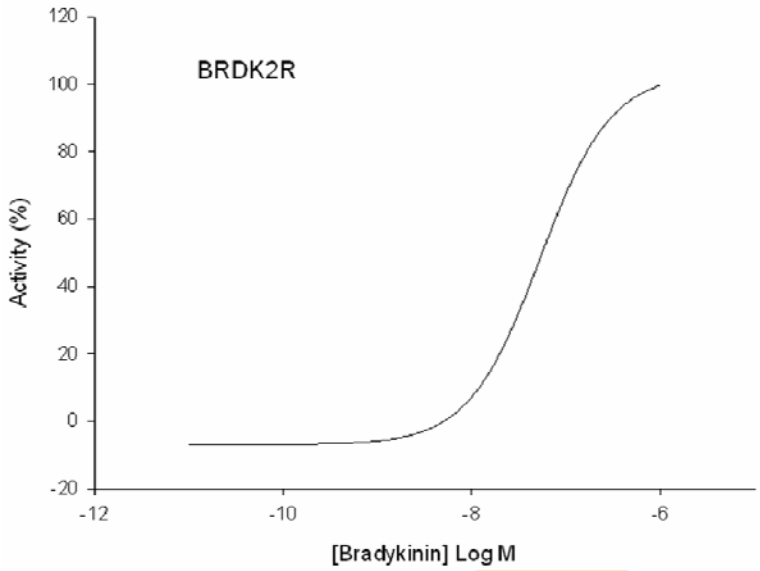
REF: P30210

RECEPTOR INTERNALIZATION ASSAYS

- FLUORESCENT BRADYKININ RECEPTOR 2 CELL LINE -





Product name: BDKR$_2$-tGFP / CHOK1 cell line

Ec$_{50}$ Bradykinin: 5.2 x 10^{-8} M

Z´: 0.63+/- 0.01

VAT No. ESB95481909

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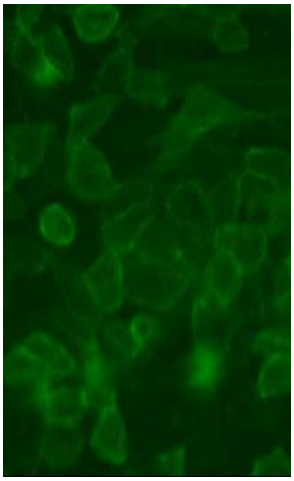


Product Name: $BDKR_2$ tGFP/CHOK1

Receptor Official Name: Human Bradykinin receptor 2

DNA Accesion Number: GenBank NM_000623

Host Cell: CHOK1

Format: 1 cryopreserved vials

References:

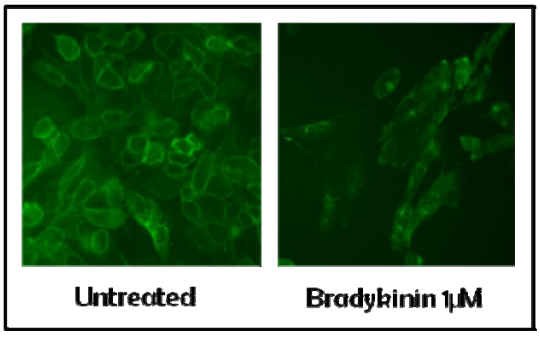
- ***P30210:*** 2 vials of 3 x 10^6 proliferative cells
- ***P30210-DA***: 1 vial of 2 x 10^6 division-arrested cells

Storage: Liquid Nitrogen

This cell line has been produced with the technology developed within FP7 PASCA EU project, and is 100% certified truly monoclonal.

Assay Briefly description

BDK2R-tGFP/CHO-K1 contains CHO-K1 cells stably expressing human Bradykinin receptor 2 (BDK2) tagged in the N-terminus with tGFP. Innoprot BDK2 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 BDKR2 activation and redistribution process in High Content Analysis and fluorescence microscope applications.

About $BDKRB_2$

Bradykinin receptor 2. BDKR2 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 2.

The 9 aa bradykinin peptide elicits many responses including vasodilation, edema, smooth muscle spasm and pain fiber stimulation. This receptor associates with G proteins, stimulates a phosphatidylinositol-calcium second messenger system.

Innoprot provides two vials of stably transfected cryopreserved CHOK1 Cells expressing recombinant human Bradykinin receptor 2 tagged in the N-terminus with tGFP (GeneBank Accesion Number: NM_000623). Each vial contains > 3 x10^6 viable cells post-thawed.

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Assay Characterization

Our expression plasmid containing the coding sequence of human Bradykinin receptor 2 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).

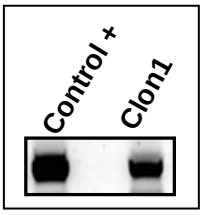

Fig.1. Clones BDK2 mRNA expression.

Activation and Internalization assay for BDK2R-tGFP

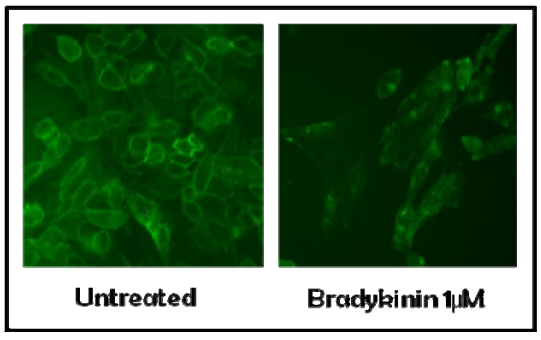

Fig.2. Internalization of BDK2-tGFP stimulated with Bradykinin. Cells were treated with 1uM Bradykinin for 6h. Activation and internalization processes were detected and analyzed using "BD Pathway 855" High-Content Bioimager from BD Biosciences.

Assay Details

CHO-K1 stably expressing human Bradykinin receptor 2 tagged in the N-terminus with tGFP were stimulated with different concentrations of Bradykinin agonist during 6 hours. After that, the nucleus was stained with DAPI and BDK2R fluorescence internalized spots were detected by fluorescence using image analysis algorithms.

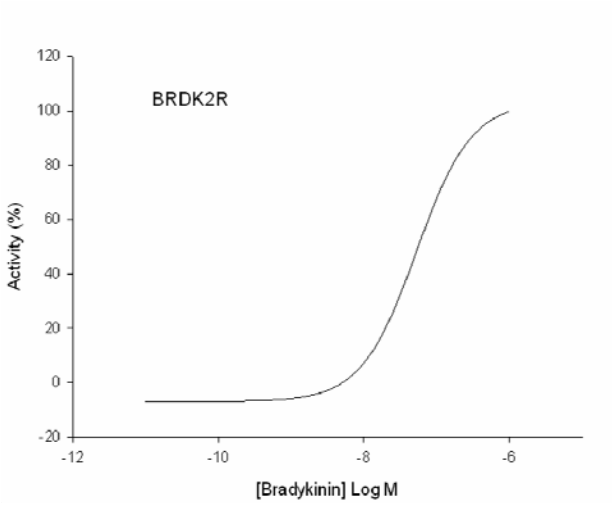

Fig.3. Bradykinin concentrations response in the BDK2R-tGFP internalization assay. Cells were treated with 8 log dilution series (n=8). The Ec50 for the Bradykinin was ~ 52nM after a treatment of 6h with agonist. Cells were fixed and the nucleus were stained with DAPI. % Activity was calculated relative to positive (1uM). The internalization assay was validated with an average of Z´=0.63+/- 0.01 for High Content Screening.

Quality Controls

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.

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