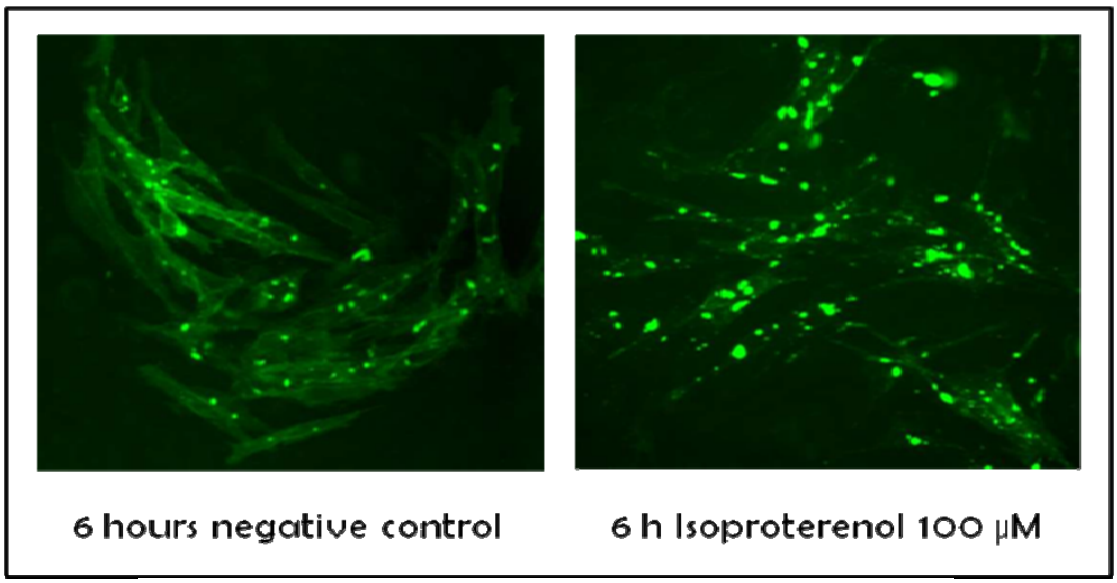
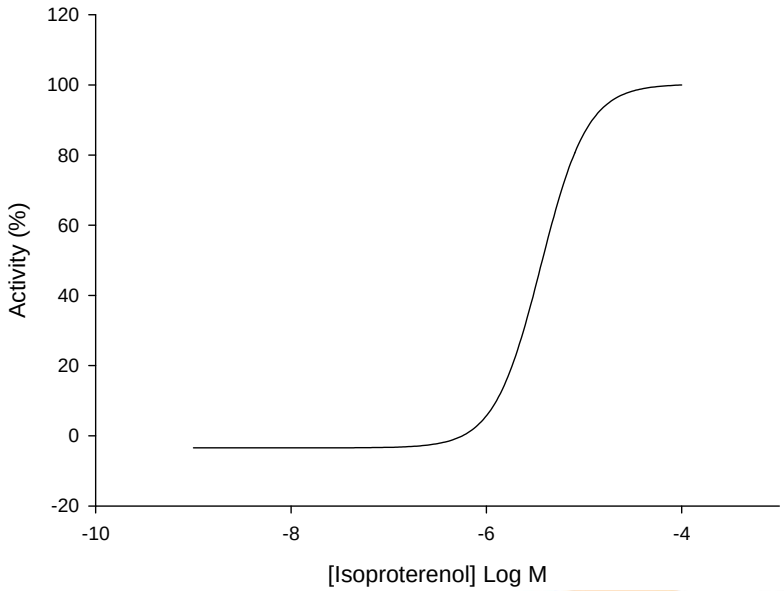
REF: P30225

RECEPTOR INTERNALIZATION ASSAYS

- FLUORESCENT ADRENERGIC RECEPTOR BETA 2 CELL LINE -





Product name: ADRB2-tGFP / CHOK1 cell line

Ec_{50} Isoproterenol: 4.0 x 10^{-6} M

Z´: 0.75 + /- 0.01

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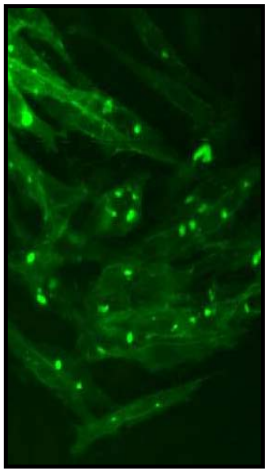


Product Name: ADRB2-tGFP/CHO-K1

Receptor Official Full Name: Human Adrenergic receptor beta 2

DNA Accesion Number: GenBank NM_000024.3

Host Cell: CHO-K1

Format: Cryopreserved vials

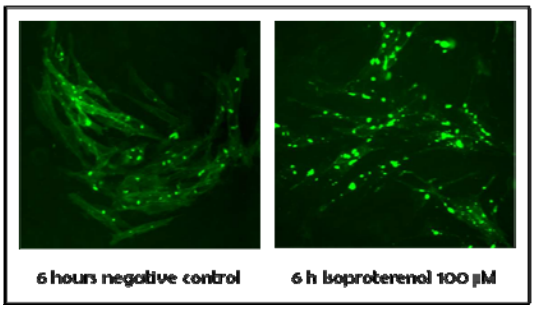
Storage: Liquid Nitrogen

References:

- ***P30225:*** 2 vials of 3 x 10^6 proliferative cells
- ***P30225-DA***: 1 vial of 2 x 10^6 division-arrested cells

Assay Briefly description

Each vial of Adrenergic receptor beta 2-tGFP/CHO-K1 contains CHO-K1 cells stably expressing human Cannabinoid receptor 2 (ADRB2) tagged in the C-terminus with tGFP. Innoprot ADRB2 redistribution Assay cells has been designed to assay compounds or analyze stimuli for their ability to modulate Adrenergic receptor beta 2 receptor activation and the following redistribution process inside the cells.



This highly reproducible assay allows monitoring ADRB2 receptor activation and redistribution process in High Content Analysis and fluorescence microscope applications.

About ADRB2 Receptor

This gene encodes beta-2-adrenergic receptor which is a member of the G protein-coupled receptor superfamily. The adrenergic receptors are a class of G protein-coupled receptors that are targets of the catecholamines, especially noradrenaline (norepinephrine) andadrenaline (epinephrine). Although dopamine is a catecholamine, its receptors are in a different category.

This receptor is directly associated with one of its ultimate effectors, the class C L-type calcium channel Ca(V)1.2. This receptor-channel complex also contains a G protein, an adenylyl cyclase, cAMP-dependent kinase, and the counterbalancing phosphatase, PP2A. Many cells possess these receptors, and the binding of an agonist will generally cause a sympathetic response (i.e. the fight-or-flight response). Different polymorphic forms, point mutations, and/or downregulation of this gene are associated with nocturnal asthma, obesity and type 2 diabetes.

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Assay Characterization

Our expression plasmid containing the coding sequence of human Adrenergic receptor beta 2 tagged in the C-terminal with tGFP protein. Our plasmid was transfected in CHO-K1 cells. Resistant clones were obtained by limit dilution, and receptor gene expression was tested by RT-PCR (Fig.1).

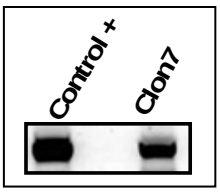

Fig.1. Clones ADRB2 mRNA expression.

Activation and Internalization assay for ADRB2-tGFP

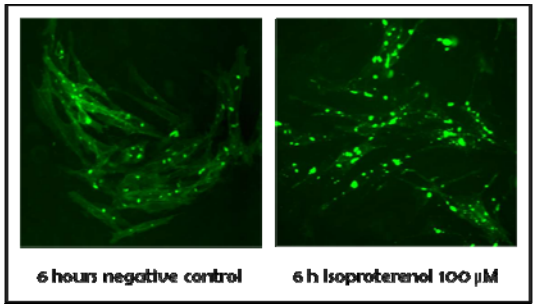

Fig.2. Redistribution of beta-2-adrenergic receptor receptor stimulated with isoproterenol. Cells were treated with 100 µM Isoproterenol for 7h. Activation and redistribution processes were detected and analyzed using "BD Pathway 855" High-Content Bioimager from BD Biosciences.

Assay Details

CHO-K1 stably expressing human beta-2-adrenergic receptor tagged in the C-terminus with tGFP were stimulated with different concentrations of Isoproterenol agonist during 7 hours. After that, the nucleus was stained with DAPI and beta-2-adrenergic receptor fluorescence redistribution was detected by fluorescence using image analysis algorithms. When cells were treated with the agonist, the human beta-2-adrenergic receptor was internalized in a big and high intensity vesicles . The activity was calculated as an increment of intensity of these vesicles.

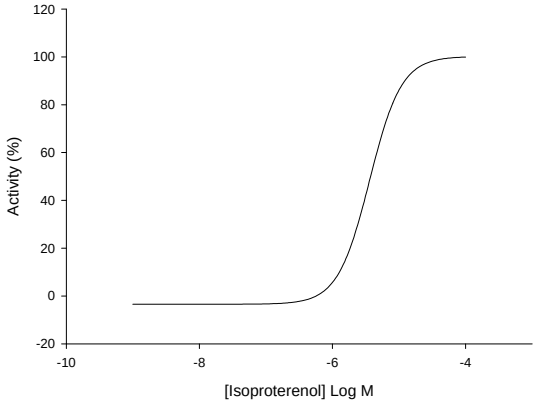

Fig.3. Isoproterenol concentrations response in the beta-2-adrenergic receptor redistribution assay. Cells were treated with 6 log dilution series (n=8). The Ec50 for the Isoproterenol was ~ 4 µM after a treatment of 7h with agonist. Cells were fixed and the nucleus were stained with DAPI. % Activity was calculated relative to positive (100 uM). The internalization assay was validated with an average of **Z´=0.75+/- 0.01** for High Content Screening.

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