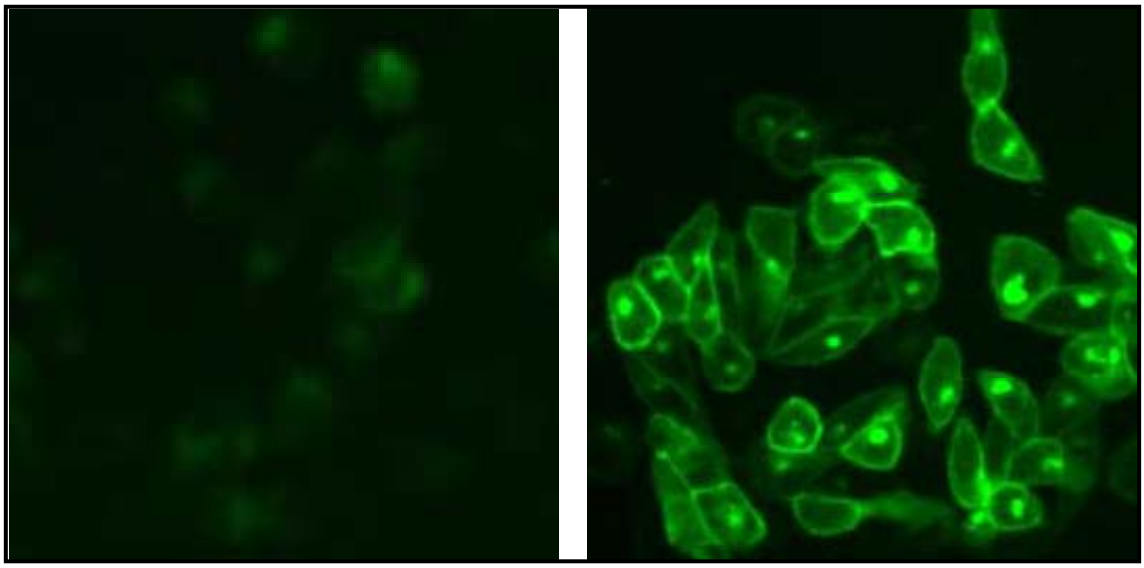
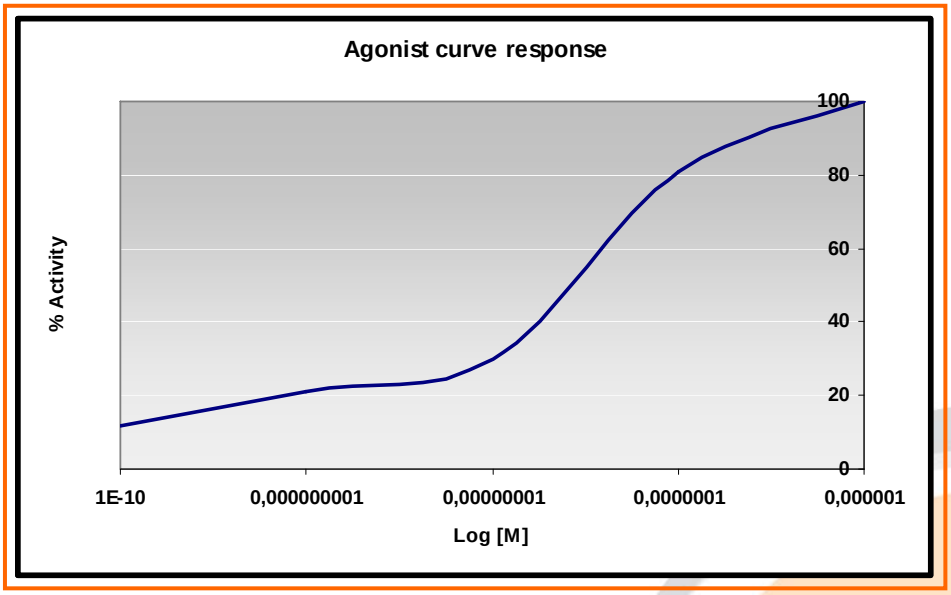
REF: P30208

RECEPTOR INTERNALIZATION ASSAYS

- FLUORESCENT CANNABINOID RECEPTOR 2 CELL LINE -





Product name: CB2-tGFP / CHOK1 cell line

Ec_{50} CP55940: 4.7 x 10^{-8} M

Z´: 0.91 + /- 0.01

VAT No. ESB95481909

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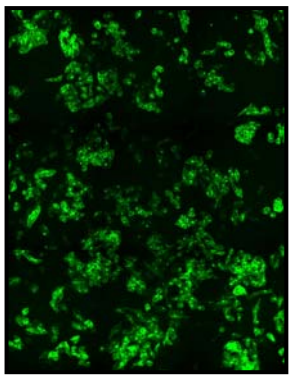
REF: P30208

RECEPTOR INTERNALIZATION ASSAYS

HUMAN CANNABINOID RECEPTOR 2 CELL LINE



Product Name: CB2R-tGFP/CHO-K1

Receptor Official Full Name: Human cannabinoid receptor 2

DNA Accesion Number: GenBank NM_001841

Host Cell: CHO-K1

Format: Cryopreserved vials

Storage: Liquid Nitrogen

References:

- ***P30208:*** 2 vials of 3 x 10^6 proliferative cells
- ***P30208-DA***: 1 vial of 2 x 10^6 division-arrested cells

Assay Briefly description

Each vial of CB2R-tGFP/CHO-K1 contains CHO-K1 cells stably expressing human Cannabinoid receptor 2 (CB2) tagged in the N-terminus with tGFP.

Innoprot CB2 internalizacion Assay has been designed to assay compounds or analyze stimuli for their ability to modulate cannabinoid receptor activation and internalization process following and quantifying the fluorescence intensity increase and distribution inside the cells.

This highly reproducible assay allows monitoring CB2 receptor activation and internalization process in High Content Analysis and fluorescence microscope applications.

About CB2 Receptor

Cannabinoid receptor 2. The cannabinoid delta-9-tetrahydrocannabinol is the principal psychoactive ingredient of marijuana. The proteins encoded by this gene and the cannabinoid receptor 1 (brain) (CNR1) gene have the characteristics of a guanine nucleotide-binding protein (G-protein)-coupled receptor for cannabinoids. They inhibit adenylate cyclase activity in a dose-dependent, stereoselective, and pertussis toxin-sensitive manner. These proteins have been found to be involved in the cannabinoid-induced CNS effects (including alterations in mood and cognition) experienced by users of marijuana. The cannabinoid receptors are members of family 1 of the G-protein-coupled receptors.

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Assay Characterization

Our expression plasmid containing the coding sequence of human Cannabinoid 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).

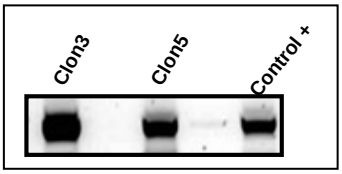

Fig.1. Clones CB2 mRNA expression.

Activation and Internalization assay for CB2R-tGFP

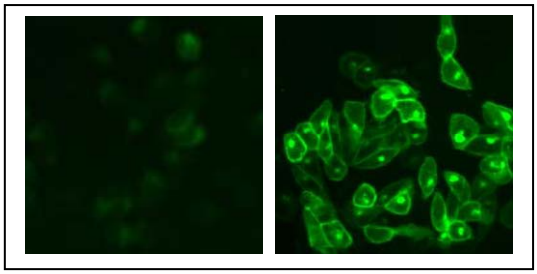

Fig.2. Internalization of CB2-tGFP clone3 and clone5 stimulated with CP-55940. Cells were treated with 100nM CP-55940 for 16h. Activation and internalization processes were detected and analyzed using "BD Pathway 855" High-Content Bioimager from BD Biosciences.

Assay Details

CHO-K1 stably expressing human Cannabinoid receptor 2 tagged in the N-terminus with tGFP were stimulated with different concentrations of CP-55940 agonist during 16 hours. After that, the nucleus was stained with DAPI and CB2R fluorescence increased and internalizated spots were detected by fluorescence using image analysis algorithms.

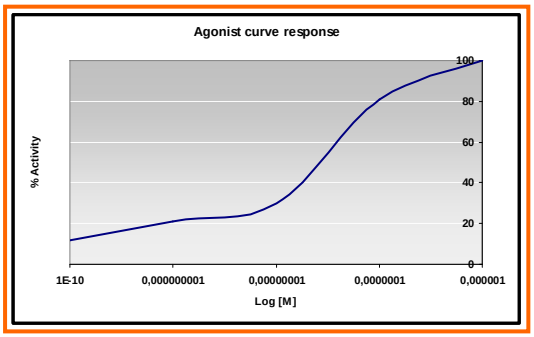

Fig.3. CP55940 concentrations response in the CB2R-tGFP internalization assay. Cells were treated with 8 log dilution series (n=8). The Ec50 for the CP55940 was ~ 47nM after a treatment of 16h with agonist. Cells were fixed and the nucleus were stained with DAPI.
% Activity was calculated relative to positive (1uM). The internalization assay was validated with an average of **Z´=0.91+/- 0.01 for High Content Screening**.

Use Restriction

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