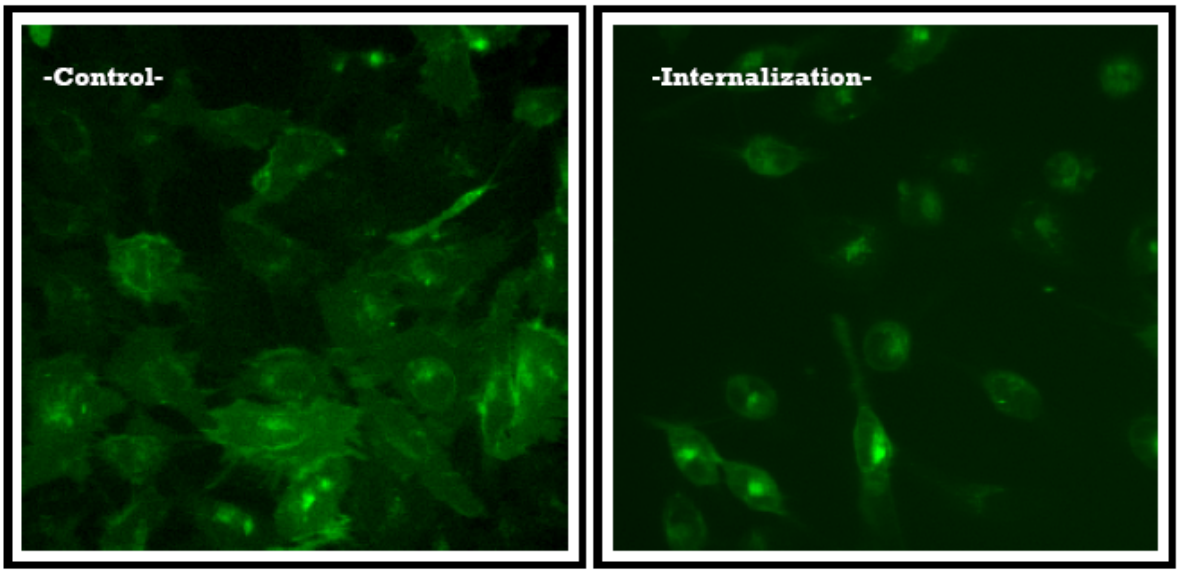
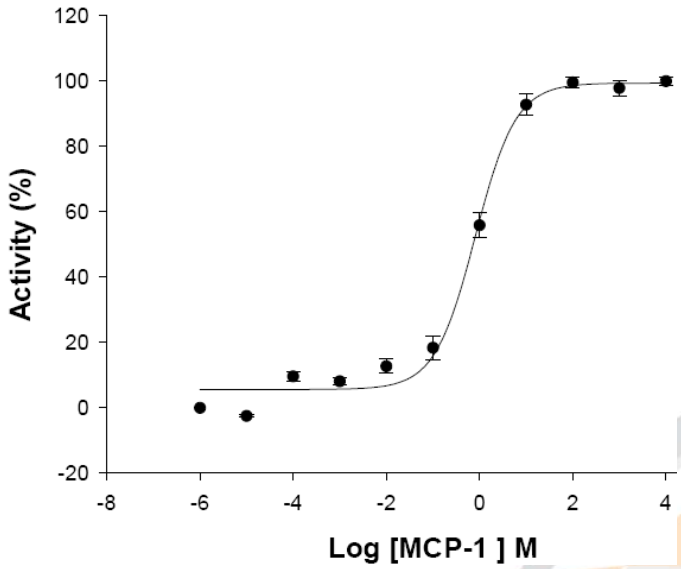
REF: P30272

RECEPTOR INTERNALIZATION ASSAYS

- FLUORESCENT HUMAN CHEMOKINE (C-C MOTIF) RECEPTOR 2 CELL LINE -





Product name: CCR2-tGFP / U2OS cell line

Ec_{50} MCP-1: 0.73 ng/ml

Z´: 0.64+/- 0.02

VAT No. ESB95481909

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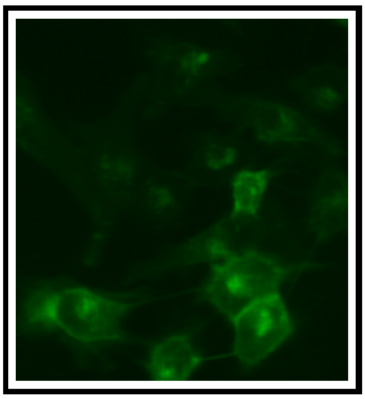

Product Name: CCR2-tGFP_U2OS

Reference: P30272

Rep. Official Full Name: Chemokine (C-C motif) receptor 2, isoform a

DNA Accession Number: Gene Bank AF545480

Host Cell: U2OS

Resistance: Puromycin

Quantity: > 3 x 10^6 cells / vial

Storage: Liquid Nitrogen

Assay Briefly description

Each vial of CCR2 Internalization Assay Cell Line contains U2OS cells stably expressing human Chemokine (C-C motif) receptor 2 (CCR2) tagged in the N-terminus with tGFP protein.

Innoprot CCR2 Internalization Assay cell line has been designed to assay potential agonists/ antagonists against CCR2, modulating its activation and the following redistribution process inside the cells. This cell line will allow the image analysis of the stimuli induced by the compounds.

This highly reproducible assay has been validated using **human MCP-1** as a CCR2 agonist in a High Content Analysis (HCA).

About chemokine (C-C motif) receptor 2

Chemokine (C-C motif) receptor 2 is a chemokine receptor. Chemokines and their receptors play roles in leukocyte recruitment and cellular functions as activation, proliferation, and differentiation.

CCR2 is expressed on the "inflammatory" subset of blood monocytes and also on other immune/inflammatory cell types such as dendritic cells and memory Th1 cells.

CCR2 is involved in the pathogenesis of animal models of RA (rheumatoid arthritis), CD (Crohn's disease), transplant rejection, atherosclerosis, and AIH (accelerated intimal hyperplasia).

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Assay Characterization

Our expression plasmid containing the coding sequence of human Chemokine (C-C motif) receptor 2 tagged in the N-terminal with tGFP protein. Our plasmid was transfected in U2OS cells. Resistant clones were obtained by limit dilution, and receptor gene expression was tested by RT-PCR (Fig.1).

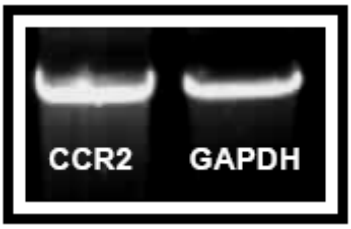

Fig1. CCR2 and GAPDH housekeeping gene RT-PCR.

Activation and Internalization assay for CCR2-tGFP (Ec50 =0.73 ng/ml)

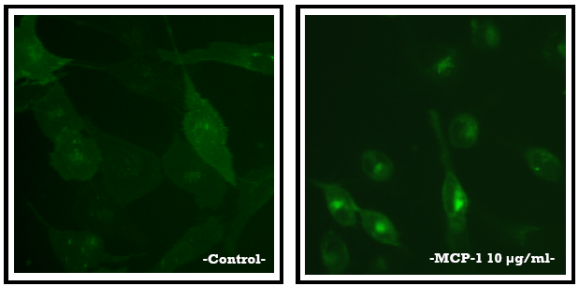

Fig2. Internalization of CCR2 stimulated with human MCP-1. Concentrations from 0 to 10 µg/ml were tested for 3h. Activation and internalization processes were detected and analyzed using "BD Pathway 855" High-Content Bioimager from BD Biosciences.

Assay Details

U2OS cells, stably expressing human Chemokine (C-C motif) receptor 2 tagged in the N-terminus with tGFP protein, were stimulated with increasing concentrations of **human MCP-1 during 3 h.** After the treatment an accumulation of fluorescence was observed around nucleus. Nuclei were stained with DAPI.

CCR2 fluorescence redistribution was determined measuring the fluorescence granularity surrounding the nuclei using image analysis algorithms.

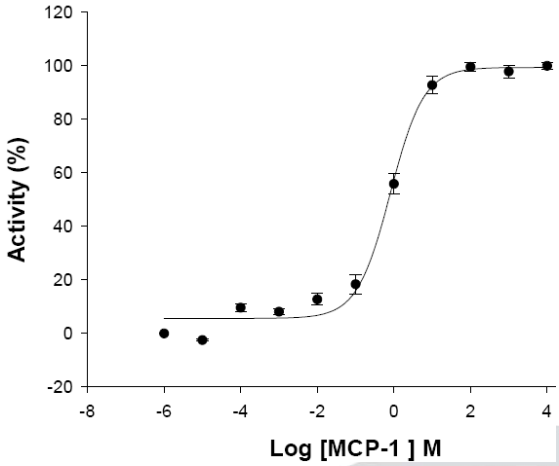

Fig3. Concentration response curve for human MCP-1 in CCR2 cell line. Cells were treated with 12 log dilution series (n=6). The Ec50 for human MCP-1 was ~ 0.73 ng/ml after a treatment of 3 h with the agonist. Cells were fixed and the nuclei were stained with DAPI. % Activity was calculated relative to positive (10 µg/ml). The internalization assay was validated with an average of Z´=**0.64+/- 0.02 f**or High Content Screening.

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