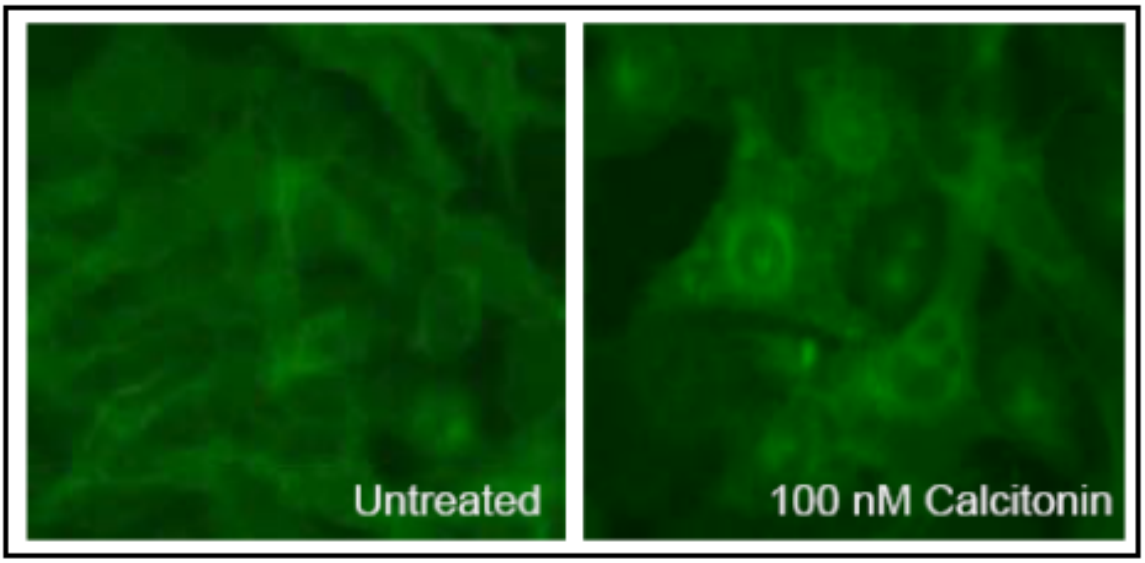
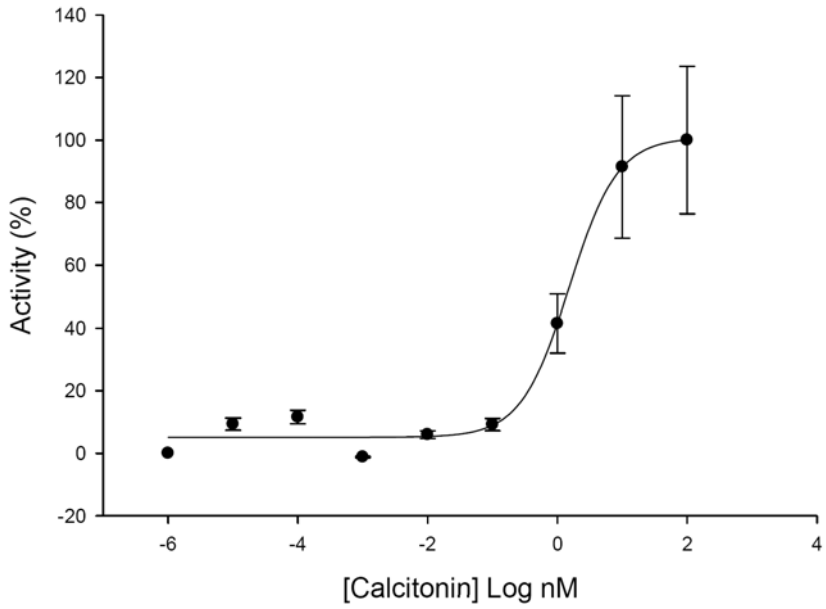
REF: P30236

RECEPTOR INTERNALIZATION ASSAYS

- FLUORESCENT CALCITONIN RECEPTOR CELL LINE -





Product name: CALCR-tGFP / CHOK1 cell line

Ec_{50} Calcitonin: 1.53 x 10^{-9} M

Z´: 0.60+/- 0.02

VAT No. ESB95481909

INNOVATIVE TECHNOLOGIES IN BIOLOGICAL SYSTEMS, S.L.
Parque Tecnológico Bizkaia, Edifício 502, 1ª Planta | 48160 | Derio | Bizkaia
Tel.: +34 944005355 | Fax: +34 946579925
innoprot@innoprot.com | www.innoprot.com

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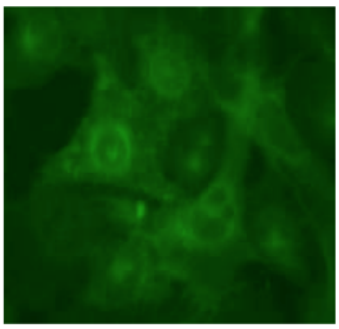


Product Name: CALCR-tGFP/CHO-K1

Receptor Official Full Name: Human Calcitonin Receptor

DNA Accesion Number: GenBank AY430048

Host Cell: CHO-K1

Format: Cryopreserved vials

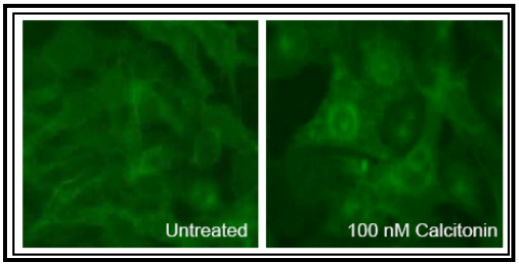
References:

- ***P30236:*** 2 vials of 3 x 10^6 proliferative cells
- ***P30236-DA***: 1 vial of 2 x 10^6 division-arrested cells

Storage: Liquid Nitrogen

Assay Briefly description

Calcitonin Receptor-tGFP/CHO-K1 contains CHO-K1 cells stably expressing human Calcitonin Receptor (CALCR) tagged in the N-terminus with tGFP. Innoprot CALCR redistribution Assay kit has been designed to assay compounds or analyze stimuli for their ability to modulate Calcitonin receptor activation and the following redistribution process inside the cells.



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

About CALCR Receptor

This gene encodes a high affinity receptor for the peptide hormone Calcitonin and belongs to a subfamily of seven transmembrane-spanning G protein coupled receptors. The protein is involved in maintaining calcium homeostasis and in regulating osteoclast-mediated bone resorption.

Polymorphisms in this gene have been associated with variations in bone mineral density and onset of osteoporosis. Alternate splicing results in multiple transcript variants.

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Assay Characterization

Our expression plasmid containing the coding sequence of human Calcitonin receptor tagged in the N-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).

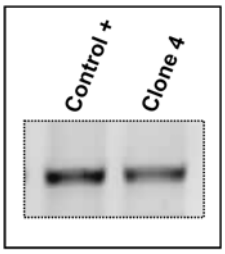

Fig.1. Clones CALCR mRNA expression.

Activation and Internalization assay for CALCR-tGFP

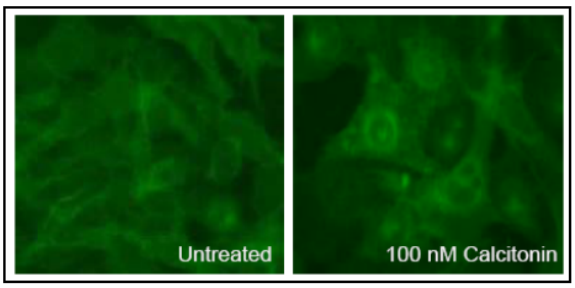

Fig.2. Internalization of CALCR stimulated with Calcitonin. Cells were treated with 100 nM Calcitonin for 3h. Activation and internalization processes were detected and analyzed using "BD Pathway 855" High-Content Bioimager from BD Biosciences.

Assay Details

CHO-K1 cells, stably expressing human Calcitonin receptor tagged in the N-terminus with tGFP, were stimulated with increasing concentrations of Calcitonin agonist during 3 hours. After the treatment the fluorescent protein was internalized in vesicles in the citosol, especially a big vesicle appeared next to the nucleus. Nuclei were stained with DAPI and Calcitonin receptor fluorescence redistribution was determined measuring the generation of the vesicle using image analysis algorithms.

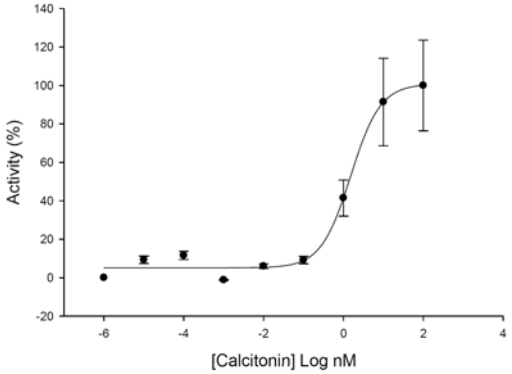

Fig.3. Concentration response curve for Calcitonin in Calcitonin receptor cell line. Cells were treated with 9 log dilution series (n=8). The Ec50 for the Calcitonin was ~ 1.53 nM after a treatment of 3 h with agonist. Cells were fixed and the nuclei were stained with DAPI. % Activity was calculated relative to positive (100 nM). The internalization assay was validated with an average of Z´=0.60+/- 0.02 for High Content Screening.

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