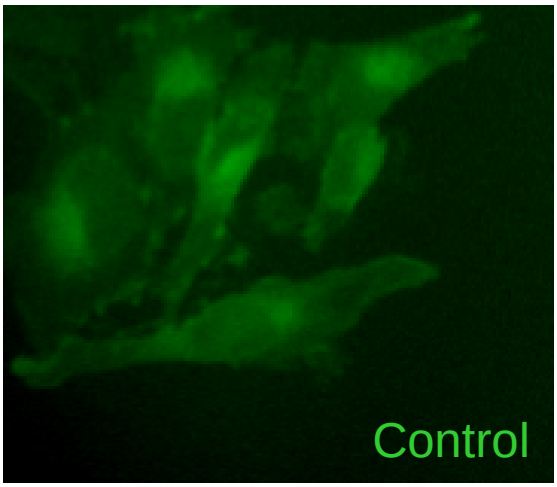
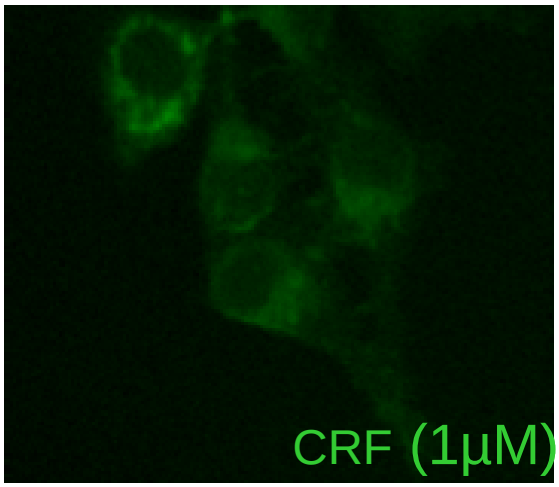
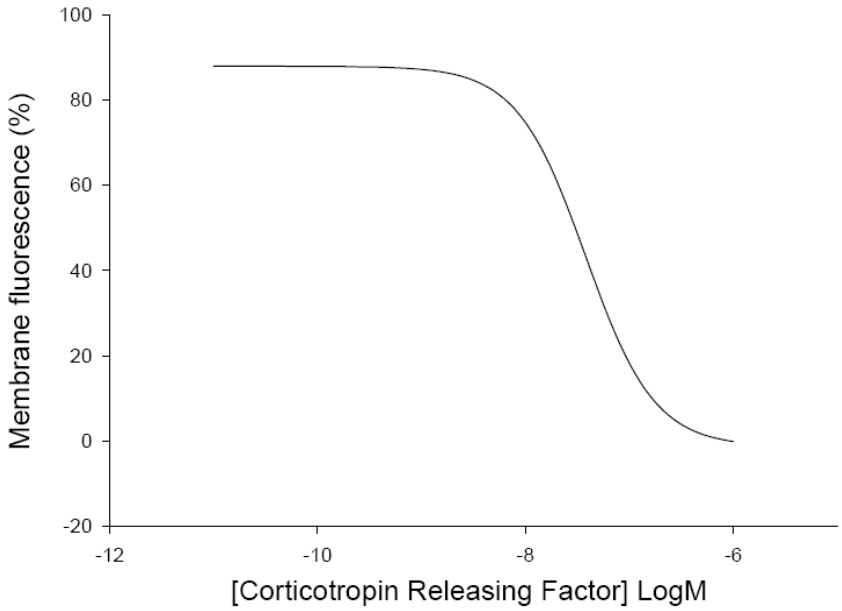
REF: P30216

RECEPTOR INTERNALIZATION ASSAYS

- CORTICOTROPIN RELEASING HORMONE RECEPTOR 2 CELL LINE -







Product name: $CRHR_2$tGFP / CHOK1 cell line

Ec_{50} CRF: 3.8 x 10^{-8} M

Z´: 0.55+/- 0.05

VAT No. ESB95481909

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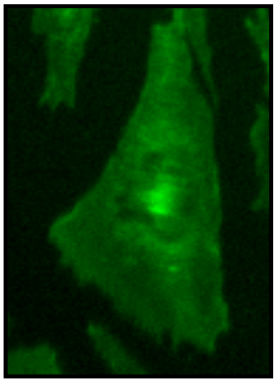


Product Name: $CRHR_2$tGFP/CHOK1

Receptor Official Name: Human Corticotropin releasing Hormone receptor 2

DNA Accesion Number: GenBank NM_001883

Host Cell: CHOK1

Format: Cryopreserved vials

References:

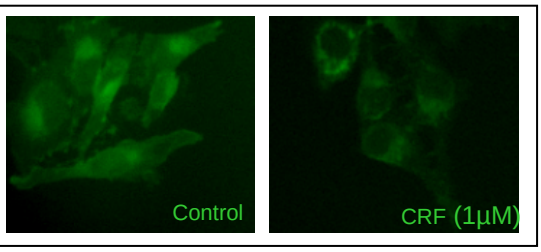
- ***P30216:*** 2 vials of 3 x 10^6 proliferative cells
- ***P30216-DA***: 1 vial of 2 x 10^6 division-arrested cells

Storage: Liquid Nitrogen

Assay Briefly description

CRHR2-tGFP/CHO-K1 contains CHO-K1 cells stably expressing human corticotropin releasing hormone receptor 2 (CRHR2) tagged in the N-terminus with tGFP.

Innoprot CRHR2 internalization Assay Cell Line has been designed to assay compounds or analyze stimuli for their ability to modulate corticotropin releasing hormone receptor 2 activation and the following internalization process quantifying the fluorescence distribution inside the cells.



This highly reproducible assay allows monitoring CRHR2 activation and internalization process in High Content Analysis and fluorescence microscope applications

About CRHR2

Corticotropin releasing hormone receptor 2 is the gene encodes a protein that is one of two Corticotropin releasing hormone receptors (CRHRs), also termed as CRFRs. The **Corticotropin releasing hormone receptor** family is a group of G-coupled receptors whose principal ligand is the corticocotropin releasing hormone (CRHR or CRF). CRH is a 41-amino acid peptide synthesized in the hypothalamus that is the principal neuroregulator of the hypothalamic-pituitary-adrenocortical axis and plays an important role in coordinating the endocrine, autonomic, and behavioural responses to stress and immune challenge.

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Assay Characterization

Our expression plasmid containing the coding sequence of human corticotropin releasing hormone 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).

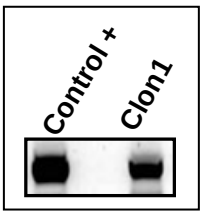

Fig.1. Clones CRHR2 mRNA expression.

Activation and Internalization assay for CRHR2-tGFP

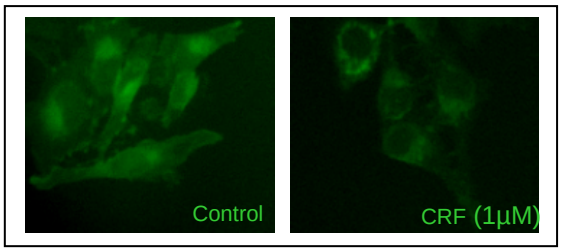

Fig.2. Internalization of CRHR2-tGFP stimulated with corticotropin hormone (CRF). Cells were treated with 1uM CFR for 6h. Activation and internalization processes were detected and analyzed using "BD Pathway 855" High-Content Bioimager from BD Biosciences.

Assay Details

CHO-K1 stably expressing human corticotrophin releasing hormone 2 tagged in the N-terminus with tGFP were stimulated with different concentrations of CRF agonist during 6 hours. After treatment the mainly membranous fluorescence pattern turned into an accumulation around the nucleus. Nuclei were stained with DAPI and CRHR2 redistribution was determined as the decrease of fluorescence in the plasmatic membrane using image analysis algorithms.

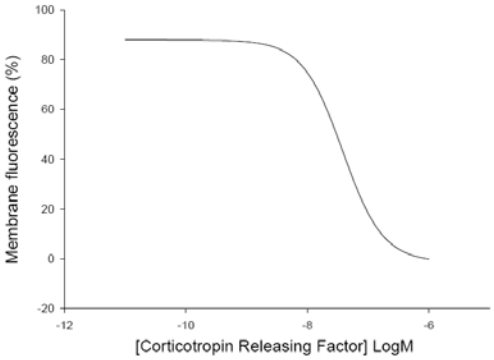

Fig.3. CRHR2-tGFP internalization in response to CRF concentrations. Cells were treated with 8 log dilution series (n=8). The Ec50 for the CRF was 38 nM after a treatment of 6h with agonist. Cells were fixed and the nuclei were stained with DAPI. % Activity was calculated relative to positive (1uM). The internalization assay was validated with an average of Z´=0.55 +/- 0.05 for High Content Screening.

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