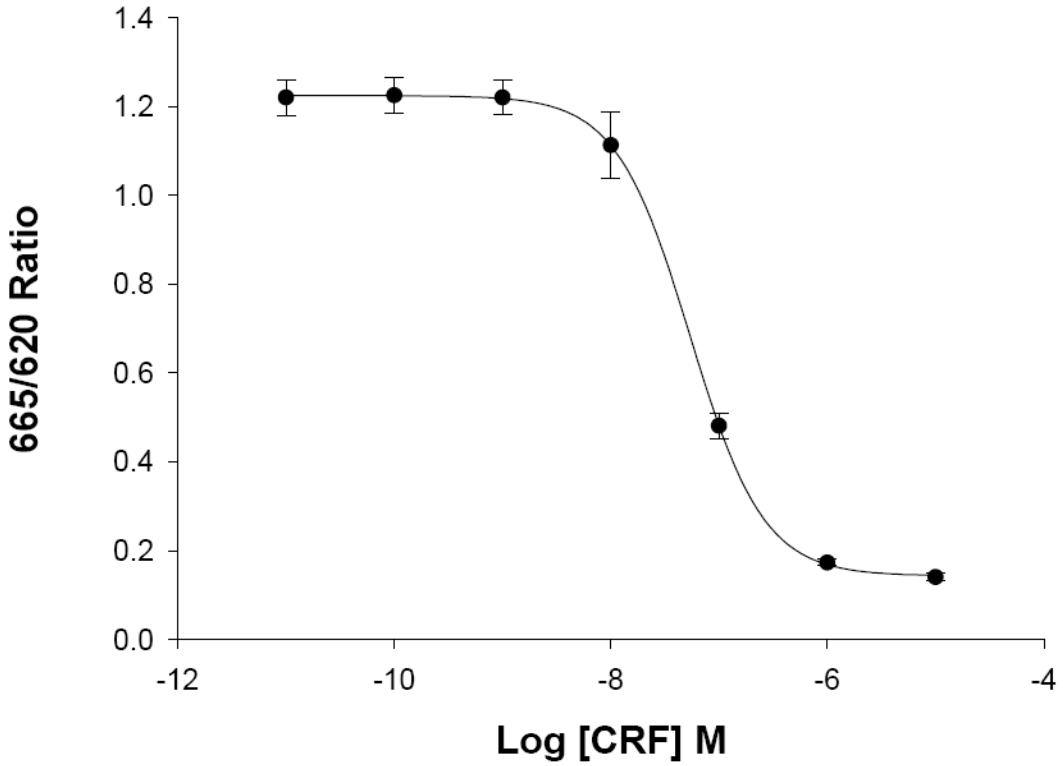
REF: P30116

HiTSeeker CELL LINES (LABEL-FREE GPCRS)

- CORTICOTROPIN RELEASING HORMONE RECEPTOR 2 CELL LINE -



Product name: CRHR-2 / HEK293 cell line

Ec_{50} CRF (Calcium Assay): 1.47 x 10^{-7} M

Z´: 0.72+/- 0.02

Ec_{50} CRF (cAMP Assay): 5.4 x 10^{-8} M

Z´: 0.86+/- 0.02

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HiTSeeker CELL LINES (LABEL-FREE GPCRS)

HUMAN CORTICOTROPIN RELEASING HORMONE RECEPTOR 2 CELL LINE

Product Name:	CRF-2 (CRHR-2)/HEK293
Official Full Name:	Corticotropin Releasing Hormone Receptor 2
DNA Accesion Number:	GenBank BC096830
Host Cell:	HEK293
Format:	2 cryopreserved vials
Resistance:	G418 (Geneticin)
Size:	*P30116*: 2 vials of 3 x 10^6 proliferative cells *P30116-DA*: 1 vial of 2.5x10^6 division-arrested cells
Storage:	Liquid Nitrogen

Assay Briefly description

CRF-2/HEK293 contains HEK293 cells stably expressing human Corticotropin Releasing Hormone Receptor 2 (CRF-2) with no tag.

Innoprot CRF-2 cell line has been designed to assay compounds or analyze their capability to modulate Corticotropin Releasing Hormone Receptor 2. When the agonist binds to CRF-2 a G protein is activated, which in turn, triggers a cellular response mediated by second messengers (Calcium and cAMP).

This cell line has been validated measuring calcium and cAMP increase in the cytosol. The high reproducibility of this assay allows monitoring CRF-2 receptor activation process in High Throughput Screening.

About CRF-2

Corticotropin Releasing Hormone Repceptor 2 is the gene that encodes a protein also known as CRHR-2. The Corticotropin Releasing Hormone Receptor family is a group of G-coupled receptors that shows high affinity for Corticotropin Releasing Hormone (CRH). CRH is a polypeptide hormone and neurotransmitter synthesized in the hypothalamus that is the principal neuroregulator of the hypothalamic-pituitary-adrenocortical axis. CRH is involved in the stress response and the stimulation of the pituitary synthesis of ACTH (Adrenocorticotropic hormone).

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Assay Characterization

Our expression plasmid contains the coding sequence of human Corticotropin Releasing Hormone Receptor protein. Our plasmid was transfected in HEK293 cells. Resistant clones were obtained by limit dilution and receptor gene expression was tested by RT-PCR using GAPDH as internal control (Fig.1).

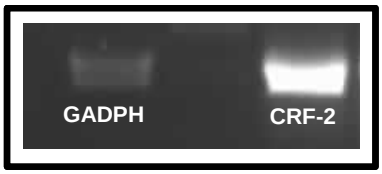

Fig.1. CRF-2 and GAPDH housekeeping gene RT-PCR.

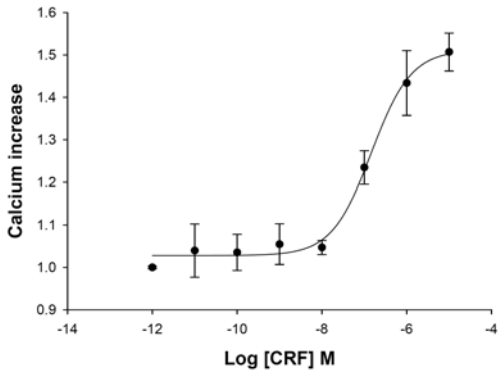

Fig.2. Corticotropin Releasing Factor dose response in calcium assay. Cells were treated with CRF concentrations ranging from 0 to $10x10^{-6}$ M (n=4). The EC_{50} for CRF was $1.47x10^{-7}$ M. The calcium assay was validated with a Z´= 0.72 for High Content Screening.

Validation of CRF-2 cell line

A) Calcium assay (Ec_{50} = $1.47x10^{-7}$ M)

A typical fluorescent calcium assay was performed using Fura-2/AM ratiometric. Calcium increase inside the cell was measured using the ratio of the fluorescence from Fura2 bound and not bound to the ion. Image acquisition was performed using a "BD Pathway 855" High-Content Bioimager from BD Biosciences.

Cells were incubated with Fura2-AM and treated with increasing Corticotropin Releasing Factor (CRF) concentrations. The receptor activity was determined by the increase of the ratio 340/380 comparing after and before the treatment.

B) cAMP production assay (Ec50= $5.4x10^{-8}$ M)

cAMP production was assessed using the cAMP dynamic 2 kit (Cisbio). This kit contains labelled cAMP (620 nm) and an anti-cAMP antibody (665nm). Between these molecules occurs a fluorescence transfer (FRET). Native cAMP produced by cells (due to the binding of an agonist to its specific receptor) competes with the labelled cAMP producing a decrease of FRET detected by HTRF technology.

The specific signal is inversely proportional to the concentration of native cAMP produced by the binding of the agonist to its receptor.

Fluorescence detection was recorded in a Multi-Mode Microplate Reader Synergy 2 from Biotek.

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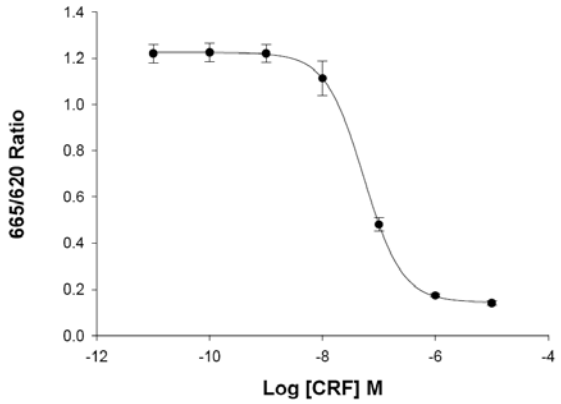

Fig.3. CRF-2 dose response curve in cAMP assay. Cells were treated with CRF concentrations ranging from 0 to $10x10^{-6}$ M (n=4). The Ec_{50} for the CRF was ~ $5.4x10^{-8}$ M. The cAMP assay was validated with an average of Z´= 0.86 for High Content Screening.

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