eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ CRF2 Corticotropin Releasing Factor Receptor Frozen Cells

CATALOG NUMBER: HTS024RTA

CONTENTS: Pack contains 2 vials of mycoplasma-free cells, 1 ml per vial. Fifty (50) mL of Media Component.

STORAGE: Vials are to be stored in liquid N_2. Media Component at 4ºC (-20ºC for prolonged storage).

BACKGROUND

Ready-to-Assay™ GPCR frozen cells are designed for simple, rapid calcium assays with no requirement for intensive cell culturing. Eurofins Discovery Services has optimized the freezing conditions to provide cells with high viability and functionality post-thaw. The user simply thaws the cells and resuspends them in media, dispenses cell suspension into assay plates and, following overnight recovery, assays for calcium response.

The corticotropin-releasing factor receptors, CRF1 and CRF2, are G_s-coupled GPCRs expressed in the brain, blood vessels and intestine that bind to several neuropeptides, including corticotropin-releasing factor (CRF) and urocortin, and the amphibian peptide sauvagine (Lovenberg *et al*., 1995; Bale and Vale, 2004). In addition, two peptides, urocortin II (Ucn II) and urocortin III (Ucn III), bind selectively and with high affinity to CRF2 (Lewis *et al*., 2001). The CRF peptides and their receptors play important roles in stress mediated by the hypothalamic-pituitary-adrenal axis in animal models, and possibly in depression and anxiety in humans, although the contributions of CRF1 and CRF2 appear to be distinct (Bale and Vale, 2004; Risbrough *et al*., 2004) In addition, CRF1 and CRF2 differentially alter feeding behavior, gastric motility and vascular tone (Zorrilla *et al*., 2004; Martinez *et al*., 2004; Wiley and Davenport, 2004). Cloned human CRF2-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant CRF2 expression on the cell surface and contains high levels of the promiscuous G protein Gα15 to couple the receptor to the calcium signaling pathway. . Thus, the cell line is an ideal tool for screening for agonists, antagonists and modulators at CRF2.

USE RESTRICTIONS

Please see User Agreement (Label License) for further details. ***One such restriction is that the contents of the supplied vial(s) are limited to a single use and shall not be propagated and/or re-frozen by licensee.***

WARNINGS

For Research Use Only; Not for Use in Diagnostic Procedures
Not for Animal or Human Consumption

GMO

This product contains genetically modified organisms.
Este producto contiene organismos genéticamente modificados.
Questo prodotto contiene degli organismi geneticamente modificati.
Dieses Produkt enthält genetisch modifizierte Organismen.
Ce produit contient organismes génétiquement des modifiés.
Dit product bevat genetisch gewijzigde organismen.
Tämä tuote sisältää geneettisesti muutettuja organismeja.
Denna produkt innehåller genetiskt ändrade organismer.

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APPLICATIONS

Calcium Flux Assays

APPLICATION DATA

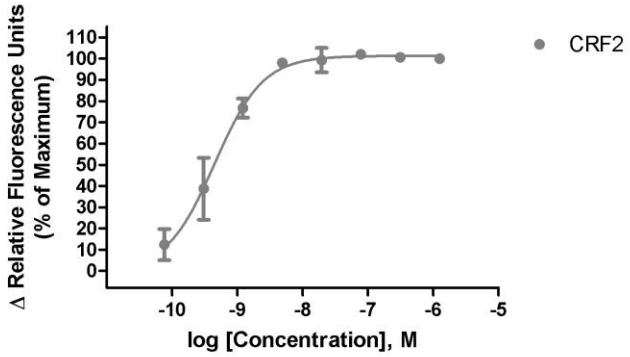

Figure 1. Representative data for activation of CRF2 receptor. Calcium flux in CRF2–expressing Chem-1 cell line induced by Urocortin. CRF2–expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 4,000 RLU (Relative Light Units).

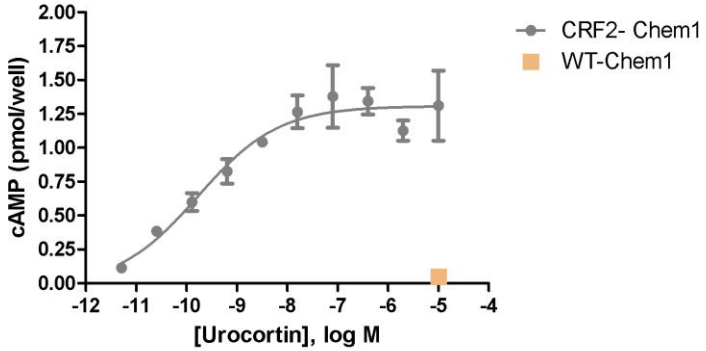

Figure 2. Representative data for activation of CRF2 receptor stably expressed in Chem-1 cells induced by Urocortin using a cAMP accumulation assay. CRF2–expressing Chem-1 cells were seeded at 100,000 cells per well into a 96-well plate, and the following day the cells were treated with Urocortin for 15 minutes in the presence of 2.0 mM IBMX and 0.5% DMSO to determine receptor-mediated cAMP generation using a time-resolved fluorescence resonance energy transfer (TR-FRET) assay measured on the BioTek Synergy. Maximal cAMP response obtained in this experiment was 1.5pmol/well. Similarly parental cells (catalog #: HTSCHEM-1) were tested to determine the specificity of the resulting signal.

Table 1. Comparison of EC_{50} values of CRF2-expressing Chem-1 cells with values described in the literature.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Urocortin	Calcium Flux	0.5	Eurofins Internal Data
Urocortin	cAMP Accumulation	0.1	Eurofins Internal Data

ASSAY SETUP

1. Immediately upon receipt, thaw cells or place cells in liquid nitrogen.
2. Thaw cells rapidly by removing from liquid nitrogen and immediately immersing in a 37ºC water bath. Immediately after ice has thawed, sterilize the exterior of the vial with 70% ethanol.
3. Add 1mL of pre-warmed Media Component to each vial of cells. Place contents from two vials into a 15 mL conical tube and bring the volume to 10 mL of Media Component.
4. Centrifuge the cell suspension at 190 x g for four minutes
5. Remove supernatant and add 10.5 mL of pre-warmed Media Component to resuspend the cell pellet.
6. Seed cell suspension into appropriate assay microplate (100 µL/well for 96-well plate, 25 µL/well for 384-well plate).
7. When seeding is complete, place the assay plate at room temperature for 30 minutes.
8. Move assay plate to a humidified 37ºC 5% CO2 incubator for 24 hours.
9. After 24 hour incubation, remove assay plate from the incubator and wash sufficiently with Hank's Balanced Salt Solution (HBSS) supplemented with 20mM HEPES, 2.5mM Probenecid at pH 7.4 to remove all trace of Media Component.
10. Prepare Fluo-8, AM (AAT Bioquest: 21080) Ca^{2+} dye by dissolving 1mg of Fluo-8 NW in 200 µL of DMSO. Once dissolved place 10 µL of Fluo-8 NW Ca^{2+} dye solution into 10 mL of HBSS 20mM HEPES, 2.5mM Probenecid pH 7.4 buffer and apply to assay microplate (Ca^{2+} dye at 10 µL /10 mL is sufficient for loading one (1) microplate).
11. Set-up FLIPR to dispense 3x ligand to appropriate wells in the assay plate. Set excitation wavelength at 470-495 nm ($FLIPR^{TETRA}$) or 485 nm (FLIPR1, FLIPR2, FLIPR3) and emission wavelength at 515-565 nm ($FLIPR^{TETRA}$) or emission filter for Ca^{2+} dyes (FLIPR1, FLIPR2, FLIPR3). Set pipet tip height to 5 µL below liquid level and dispense rate to 75 µL/sec (96-well format) or 50 µL/sec (384-well format). Set up plate layout and tip layout for each individual experiment. Set time course for 180 seconds, with ligand addition at 10 seconds.
12. Ligands are prepared in non-binding surface Corning plates (Corning 3605 – 96-well or Corning 3574 – 384-well).
13. After the run is complete, negative control correction is applied and data analyzed utilizing the maximum statistic.

ASSAY MATERIALS

Description	Supplier and Product Number
HBSS	Hyclone: SH30268.02
HEPES 1M Stock	EMD Millipore.: TMS-003-C
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
Urocortin ligand	Tocris: 1604
Non-binding white plates (for ligand prep)	Corning: 3605(96-well)/3574(384-well)
Black (clear bottom) tissue-culture treated plates	Corning: 3904(96-well)/3712(384-well)

FLIPR SETTINGS

Settings for FLIPR^{TETRA}® with ICCD camera option

Option	Setting
Read Mode	Fluorescence
Ex/Em	Ex470_495 / Em515_575
Camera Gain	2000
Gate Open	6 %
Exposure Time	0.53
Read Interval	1s
Dispense Volume	50 µl (25 µl for 384-well)
Dispense Height	25 µl (50 µl for 384-well)
Dispense Speed	75 µl L/sec (50 µl for 384-well)
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

HOST CELL

Chem-1, an adherent rat hematopoietic cell line expressing endogenous Gα15 protein.

EXONGENOUS GENE EXPRESSION

CRF2 cDNA (Accession Number: NM_001883; see CODING SEQUENCE below) expressed from a proprietary expressed from a proprietary pHS plasmid.

RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS024M	ChemiScreen™ CRF2 CRF receptor membrane prep

REFERENCES

1. Bale TL and Vale WW (2004) CRF and CRF receptors: role in stress responsivity and other behaviors. *Annu. Rev. Pharmacol. Toxicol.* 44: 525-557.
2. Lewis K *et al.* (2001) Identification of urocortin III, an additional membrer of the corticotropin-releasing factor (CRF) family with high affinity for the CRF2 receptor. *Proc. Natl. Acad. Sci. USA* 98: 7570-7575.
3. Lovenberg TW *et al.* (1995) Cloning and characterization of a functionally distinct corticoptropin-releasing factor receptor subtype from rat brain. *Proc. Natl. Acad. Sci. USA 92: 836-840*.
4. Martinez V *et al.* (2004) Central CRF, urocortins and stress increase colonic transit via CRF1 receptors while activation of CRF2 receptors delays gastric transit in mice. *J. Physiol.* 556: 221-234.
5. Risbrough VB *et al.* (2004) Corticotropin-releasing factor receptors CRF1 and CRF2 exert both additive and opposing influences on defensive startle behavior. *J. Neurosci.* 24: 6545-6552.
6. Wiley KE and Davenport AP (2004) CRF2 receptors are highly expressed in the human cardiovascular system and their cognate ligands urocortins 2 and 3 are potent vasodilators. *Br. J. Pharmacol.* 143: 508-514.

User Agreement (Label License)

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