eurofins | Discovery Services

PRODUCT DATASHEET

ChemiScreen™ CRF_2 Corticotropin Releasing Factor Receptor Stable Cell Line

CATALOG NUMBER: HTS024C

CONTENTS: 2 vials of mycoplasma-free cells, 1 mL per vial.

STORAGE: Vials are to be stored in liquid N_2.

BACKGROUND

ChemiScreen cell lines are constructed in the Chem-1 host, which supports high levels of functional receptor expression on the cell surface. Chem-1 cells contain high endogenous levels of Gα15, a promiscuous G protein, allowing most receptors to couple to the calcium signaling pathway.

The corticotropin-releasing factor receptors, CRF_1 and CRF_2, 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 CRF_2 (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 CRF_1 and CRF_2 appear to be distinct (Bale and Vale, 2004; Risbrough *et al*., 2004) In addition, CRF_1 and CRF_2 differentially alter feeding behavior, gastric motility and vascular tone (Zorrilla *et al*., 2004; Martinez *et al*., 2004; Wiley and Davenport, 2004). The cloned human CRF_2-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant CRF_2 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 antagonists of interactions between CRF_2 and its ligands.

USE RESTRICTIONS

Please see **Limited Use Label License Agreement** (Label License Agreement) for further details.

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.

Eurofins Pharma Bioanalytics Services US Inc.
6 Research Park Drive
St Charles MO 63304
USA
T | +1 844 522 7787
F | +1 636 362 7131
www.eurofins.com

APPLICATIONS

cAMP accumulation

APPLICATION DATA

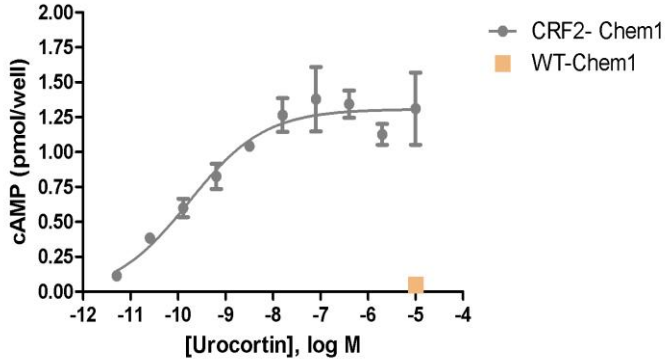

Figure 1. Representative data for activation of CRF_2 receptor stably expressed in Chem-1 cells induced by Urocortin using a cAMP accumulation assay. CRF_2–expressing Chem-1 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were treated with Urocortin for 10 minutes in the presence of 100 µM IBMX and 0.5% DMSO to determine receptor-mediated cAMP generation or [with Urocortin for 10 minutes in the presence of 100 µM IBMX and 10 µM forskolin, to stimulate adenylate cyclase, and 0.5% DMSO to determine the receptor's ability to inhibit cAMP generation using a time-resolved fluorescence resonance energy transfer (TR-FRET) assay measured on the BioTek Synergy. Maximal fluorescence signal obtained in this experiment was 7,000 RLU. Similarly parental cells (catalog #: HTSCHEM-1) were tested to determine the specificity of the resulting signal.

Table 1. EC_{50} value of CRF_2-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
Urocortin	cAMP accumulation	0.1	Eurofins Internal Data

* The cell line was tested and found to have equivalent EC_{50} and signal at 1, 3 and 6 weeks of continuous culture by calcium flux fluorescence.

CELL CULTURE

Table 2. Recommended Cell Culture Reagents (not provided)

Description	Component	Concentration	Supplier and Product Number
Basal Medium	DMEM high glucose Medium (4.5g/L)	-	Hyclone: SH30022
	Fetal Bovine Serum (FBS)	10%	Hyclone: SH30070.03
	Non-Essential Amino Acids (NEAA)	1X	Hyclone: SH30238.01
	HEPES	1X	EMD Millipore: TMS-003-C
Selection Medium	Basal Medium (see above)	-	
	Geneticin (G418)	250 µg/ml	Invivogen: ant-gn-5
Dissociation	Sterile PBS	-	Hyclone: SH30028.03
	0.25% Trypsin-EDTA	-	Hyclone: SH30042.01
CryoMedium	Basal Medium (see above)	40%	
	Fetal Bovine Serum (FBS)	50%	Hyclone: SH30070.03
	Dimethyl Sulfoxide (DMSO)	10%	Sigma: D2650

Cell Handling

1. Upon receipt, directly place cells in liquid nitrogen storage. Consistent cryopreservation is essential for culture integrity.
2. Prepare Basal Medium. Prepare 37ºC Water Bath. Thaw cells rapidly by removing from liquid nitrogen, and immediately immersing in a 37ºC water bath, until 90% thawed. Immediately sterilize the exterior of the vial with 70% ethanol.
3. Add vial contents to 15 mL Basal Medium in T75 Tissue Culture Treated Flask. Gently swirl flask and place in a humidified, tissue culture incubator, 37ºC, 5% CO_2.
4. 18-24 Hours Post–Thaw, all live cells should be attached. Viability of the cells is expected to be 60-90%, At this time, exchange Basal Medium with Selection Medium.
5. When cells are approximately 80% confluent, passage the cells. It is suggested that user expand culture to create >20 vial Master Cell Bank at low passage number. *Cells should be maintained at less than 80% confluency for optimal assay results.*
6. Cell Dissociation: Aspirate Culture Medium. Gently wash with 1x Volume PBS. Add 0.1x Volume Warm Trypsin-EDTA. Incubate 4 min, 37°C, until cells dislodge. *If cells do not round up, place in 37º C incubator for additional 2 min*. Neutralize Trypsin and collect cells in 1x Volume Basal Medium.
7. Seed Cells for expansion of culture. It is recommended that cell lines are passaged at least once before use in assays.

Table 3. Cell Culture Seeding Suggestions: *User should define based on research needs.*

Flask Size (cm^2)	Volume (mL)	Total Cell Number ($x10^6$)	Growth Period (hrs)
T75	15	5.0	24
T75	15	2.0	48
T75	15	0.45	72

ASSAY SETUP

Fluorescence

Table 4. 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	95 µl (50 µl for 384-well)
Dispense Speed	50 µl/sec
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

Table 5. Assay Materials (Not provided)

Description	Supplier and Product Number
HBSS	Invitrogen: 14025
HEPES 1M Stock	EMD Millipore: TMS-003-C
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
Urocortin ligand	Tocris: 1604
Non-Binding 96/384 well Plates (for ligand prep)	Corning: 3605/ 3574
Black (clear Bottom) cell assay plates	Corning: 3904/ 3712
Coelenterazine-h (250µg). Prepare to 10mM	Promega: S2011

Assay Protocol – Fluorescence

1. Dissociate Culture as Recommended. Collect in Basal Medium. Document Cell Count and Viability
2. Centrifuge the cell suspension at 190 x g for six min
3. Remove supernatant. Gently resuspend the cell pellet in Basal Medium. *It is suggested that end user optimize cell plating based on individual formats.* (Default: Resuspend in volume to achieve $5x10^5$cells/ml *(i.e, if collected 5e6 TC,* $^{5e6/}_{5e5/ml}$ *=10 mL volume)*
4. Seed cell suspension into black, clear bottom plate (100 µL/well for 96-well plate). *When seeding is complete, place the assay plate at room temperature for 30 min.*
5. Move assay plate to a humidified 37ºC 5% CO_2 incubator for 18-24 h.
6. Next day, prepare Assay buffer (HBSS, 20mM HEPES, 2.5 mM Probenicid, pH 7.4) and Loading buffer (Assay buffer with 5 mM Fluo8 Dye). *Note: Please prepare Fluo8 stock according to Manufacturer's Recommendations*
7. Remove medium from assay plate and wash 1X with Assay Buffer.
8. Add Loading buffer to assay plate (100 µL/well for 96-well plate). Incubate plate for 1.5 h at room temperature, protected from light.
9. Prepare ligands in assay buffer at 3x final concentration in non-binding plates. Use Buffer Only Control Wells for Background Subtraction.
10. Create protocol for ligand addition. Please refer to FLIPR^{TETRA}® settings provided in Table 2. Set time course for 180 s, with ligand addition at 10 s.
11. After the run is complete, apply subtract bias on sample 1. We recommend using Negative Control Correction with Buffer Only Wells. Export data to according to research needs. For most Calcium Flux analysis using Export of Max Signal to end of run is sufficient.

HOST CELL

Chem-1, an adherent cell line expressing the promiscuous G-protein, Gα15.

EXOGENOUS GENE EXPRESSION

Human CRF_2 cDNA (Accession Number: NM_001883) and promiscuous G protein are expressed in a bicistronic vector

RELATED PRODUCTS

Product Number	Description
HTSCHEM-1	ChemiScreen™ Chem-1 Parental Cell Line (control cells)
HTS024M	ChemiScreen™ CRF_2 Corticotropin Releasing Factor 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 .
7. Zorrilla EP *et al.* (2004) Human urocortin 2, a corticotropin-releasing factor (CRF)2 agonist, and ovine CRF, a CRF1 agonist, differentially alter feeding and motor activity. *J. Pharmacol. Exp. Ther.* 310: 1027-1034.

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION

Limited Use Label License Agreement

In addition to the General Terms & Conditions of Sale for Products and Services section, this Product is subject to Limited Use Label License Agreement. Please go to https://www.eurofinsdiscoveryservices.com/cms/cms-content/misc/legal-disclaimer/ for more information.