eurofins | Discovery Services

PRODUCT DATASHEET

ChemiScreen™ CCK_1 Cholecystokinin Receptor Stable Cell Line

CATALOG NUMBER: HTS184C

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.

Cholecystokinins are a series of peptides of heterogeneous length (5 to 58 amino acids) that are derived from preprocholecystokinin and are found in gastrointestinal tissues and the central nervous system. Gastrin is a related peptide with 5 C-terminal amino acids identical to those of cholecystokinin. Two GPCRs, CCK_1 (CCK_A) and CCK_2 (CCK_B), bind to CCK and/or gastrin to mediate the biological effects of the peptides. CCK_1 selectively binds sulfated CCK, whereas CCK_2 binds to CCK and gastrin with similar affinity. Binding of ligands to CCK_1 stimulates mobilization of intracellular calcium by activation of $G_{q/11}$. CCK_1 receptors in the periphery are primarily localized in the pancreas, gallbladder, pylorus, and intestine where they are responsible of the regulation of diverse digestive processes. They are also present in select areas of the peripheral nervous system (vagus nerve), and the CNS where they mediate the satiety effects of CCK, regulate an increase in dopamine release, and antagonize opiod analgesia (Noble *et al.*, 1999). The cloned human CCK_1-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant CCK_1 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 the CCK_1 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.

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APPLICATIONS

Calcium Flux Fluorescence Assay

APPLICATION DATA

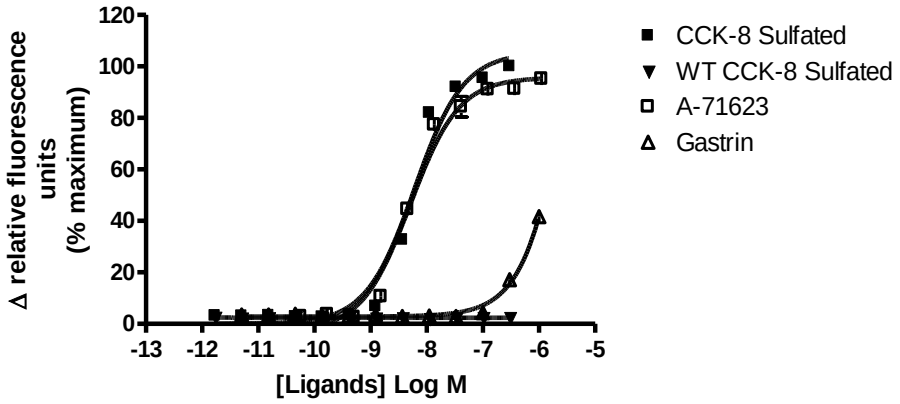

Figure 1. Representative data for activation of CCK_1 receptor stably expressed in Chem-1 cells induced by CCK-8 Sulfated using a fluorescent calcium flux assay. CCK_1–expressing Chem-1 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a fluorescent calcium indicator. Calcium flux in response to the indicated ligand with a final concentration of 0.5% DMSO was determined on a Molecular Devices FLIPR^{TETRA}® with ICCD camera. Maximal fluorescence signal obtained in this experiment was 6,000 RLU. Similarly parental cells (catalog #: HTSCHEM-1) were tested to determine the specificity of the resulting signal.

Table 1. EC_{50} value of CCK_1-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
CCK-8 Sulfated	Calcium Flux - Fluorescence	5	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
CCK-8 Sulfated	Sigma: C2175
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 CCK_1 cDNA (Accession Number: NM_000730; see CODING SEQUENCE below) and promiscuous G protein are expressed in a bicistronic vector

CODING SEQUENCE

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                                                ATG GAT GTG GTT GAC AGC CTT CTT GTG AAT GGA AGC
                                                 M   D   V   V   D   S   L   L   V   N   G   S

AAC ATC ACT CCT CCC TGT GAA CTC GGG CTC GAA AAT GAG ACG CTT TTC TGC TTG GAT CAG CCC CGT CCT
 N   I   T   P   P   C   E   L   G   L   E   N   E   T   L   F   C   L   D   Q   P   R   P

TCC AAA GAG TGG CAG CCA GCG GTG CAG ATT CTC TTG TAC TCC TTG ATA TTC CTG CTC AGC GTG CTG GGA
 S   K   E   W   Q   P   A   V   Q   I   L   L   Y   S   L   I   F   L   L   S   V   L   G

AAC ACG CTG GTC ATC ACC GTG CTG ATT CGG AAC AAG CGG ATG CGG ACG GTC ACC AAC ATC TTC CTC CTC
 N   T   L   V   I   T   V   L   I   R   N   K   R   M   R   T   V   T   N   I   F   L   L

TCC CTG GCT GTC AGC GAC CTC ATG CTC TGT CTC TTC TGC ATG CCG TTC AAC CTC ATC CCC AAT CTG CTC
 S   L   A   V   S   D   L   M   L   C   L   F   C   M   P   F   N   L   I   P   N   L   L

AAG GAT TTC ATC TTC GGG AGC GCC GTT TGC AAG ACC ACC ACC TAC TTC ATG GGC ACC TCT GTG AGT GTA
 K   D   F   I   F   G   S   A   V   C   K   T   T   T   Y   F   M   G   T   S   V   S   V

TCT ACC TTT AAT CTG GTA GCC ATA TCT CTA GAG AGA TAT GGT GCG ATT TGC AAA CCC TTA CAG TCC CGG
 S   T   F   N   L   V   A   I   S   L   E   R   Y   G   A   I   C   K   P   L   Q   S   R

GTC TGG CAG ACA AAA TCC CAT GCT TTG AAG GTG ATT GCT GCT ACC TGG TGC CTT TCC TTT ACC ATC ATG
 V   W   Q   T   K   S   H   A   L   K   V   I   A   A   T   W   C   L   S   F   T   I   M

ACT CCG TAC CCC ATT TAT AGC AAC TTG GTG CCT TTT ACC AAA AAT AAC AAC CAG ACC GCG AAT ATG TGC
 T   P   Y   P   I   Y   S   N   L   V   P   F   T   K   N   N   N   Q   T   A   N   M   C

CGC TTT CTA CTG CCA AAT GAT GTT ATG CAG CAG TCC TGG CAC ACA TTC CTG TTA CTC ATC CTC TTT CTT
 R   F   L   L   P   N   D   V   M   Q   Q   S   W   H   T   F   L   L   L   I   L   F   L

ATT CCT GGA ATT GTG ATG ATG GTG GCA TAT GGA TTA ATC TCT TTG GAA CTC TAC CAG GGA ATA AAA TTT
 I   P   G   I   V   M   M   V   A   Y   G   L   I   S   L   E   L   Y   Q   G   I   K   F

GAG GCT AGC CAG AAG AAG TCT GCT AAA GAA AGG AAA CCT AGC ACC ACC AGC AGC GGC AAA TAT GAG GAC
 E   A   S   Q   K   K   S   A   K   E   R   K   P   S   T   T   S   S   G   K   Y   E   D

AGC GAT GGG TGT TAC CTG CAA AAG ACC AGG CCC CCG AGG AAG CTG GAG CTC CGG CAG CTG TCC ACC GGC
 S   D   G   C   Y   L   Q   K   T   R   P   P   R   K   L   E   L   R   Q   L   S   T   G

AGC AGC AGC AGG GCC AAC CGC ATC CGG AGT AAC AGC TCC GCA GCC AAC CTG ATG GCC AAG AAA AGG GTG
 S   S   S   R   A   N   R   I   R   S   N   S   S   A   A   N   L   M   A   K   K   R   V

ATC CGC ATG CTC ATC GTC ATC GTG GTC CTC TTC TTC CTG TGC TGG ATG CCC ATC TTC AGC GCC AAC GCC
 I   R   M   L   I   V   I   V   V   L   F   F   L   C   W   M   P   I   F   S   A   N   A

TGG CGG GCC TAC GAC ACC GCC TCC GCA GAG CGC CGC CTC TCA GGA ACC CCC ATT TCC TTC ATC CTC CTC
 W   R   A   Y   D   T   A   S   A   E   R   R   L   S   G   T   P   I   S   F   I   L   L

CTG TCC TAC ACC TCC TCC TGC GTC AAC CCC ATC ATC TAC TGC TTC ATG AAC AAA CGC TTC CGC CTC GGC
 L   S   Y   T   S   S   C   V   N   P   I   I   Y   C   F   M   N   K   R   F   R   L   G

TTC ATG GCC ACC TTC CCC TGC TGC CCC AAT CCT GGT CCC CCA GGG GCG AGG GGA GAG GTG GGG GAG GAG
 F   M   A   T   F   P   C   C   P   N   P   G   P   P   G   A   R   G   E   V   G   E   E

GAG GAA GGC GGG ACC ACA GGA GCC TCT CTG TCC AGG TTC TCG TAC AGC CAT ATG AGT GCC TCG GTG CCA
 E   E   G   G   T   T   G   A   S   L   S   R   F   S   Y   S   H   M   S   A   S   V   P

CCC CAG TGA
 P   Q  Stp
```

RELATED PRODUCTS

Product Number	Description
HTSCHEM-1	ChemiScreen™ Chem-1 Parental Cell Line (control cells)
HTS184M	ChemiScreen™ CCK_1 Cholecystokinin Receptor Membrane Prep

REFERENCES

1. Noble F *et al.* (1999) International Union of Pharmacology. XXI. Structure, distribution, and functions of cholecystokinin receptors. *Pharmacol. Rev.* 51: 745-781.

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