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PRODUCT DATASHEET

Ready-to-Assay™ CCK1 Cholesystokinin Receptor Frozen Cells

CATALOG NUMBER: HTS184RTA

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.

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). 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 for functional detection via the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists, antagonists, and modulators at CCK_1.

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

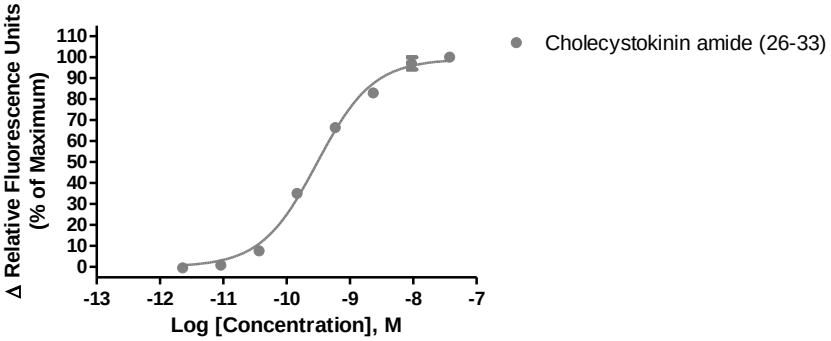

Figure 1. Representative data for activation of CCK1 receptor. Calcium flux in CCK1–expressing Chem-1 cell line induced by Cholecystokinin amide (26-33). CCK1–expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 5,500 RLU (Relative Light Units).

Table 1. EC_{50} values of CCK1-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Cholecystokinin amide (26-33)	Calcium Flux	0.3	Eurofins Internal Data
Gastrin I (not shown)	Calcium Flux	>1,000	Eurofins Internal Data
A-71623 (not shown)	Calcium Flux	5.1	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
Cholecystokinin amide (26-33) ligand	Sigma: C2175
Gastrin I ligand	Bachem: H-3085
A-71623 ligand	
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

Human CCKAR cDNA (Accession Number: NM_000730; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

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
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS184M	ChemiScreen™ CCK1 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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