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PRODUCT DATASHEET

ChemiScreen™ APJ Apelin Receptor Stable Cell Line

CATALOG NUMBER: HTS068C

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-5 host, which supports high levels of functional receptor expression on the cell surface. Chem-5 cells contain high endogenous levels of Gα15, a promiscuous G protein, allowing most receptors to couple to the calcium signaling pathway.

Apelin peptides have been discovered to be a family of peptides of different sizes that is derived from the N-terminus of a 77 amino acid precursor peptide (preproapelin) (Hosoya *et al.*, 2000). Apelin receptor (APJ) is a G protein-coupled receptor that is activated by several apelin fragments, which results in inhibition of cAMP production (Habata *et al*., 1999). APJ and apelin peptides have been found to be involved in the regulation of cardiovascular function (Katugampola *et al*., 2001) and fluid homeostasis (Reaux *et al*., 2001). Broad roles of apelin system has been established in lowering blood pressure, as a potent cardiac inotrope, in modulating pituitary hormone release and food and water intake, in stress activation, and as a novel adipokine that is excreted from fat cells and regulates insulin (Lee *et al*., 2006). The cloned human APJ receptor-expressing ChemiScreen cells were constructed by stable transfection of Chem-5 cells with receptor and a promiscuous G protein to couple the receptor to the calcium signaling pathway. These stability-tested cells are ready for fluorescence-based assays for agonists, antagonists and modulators at the APJ receptor.

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

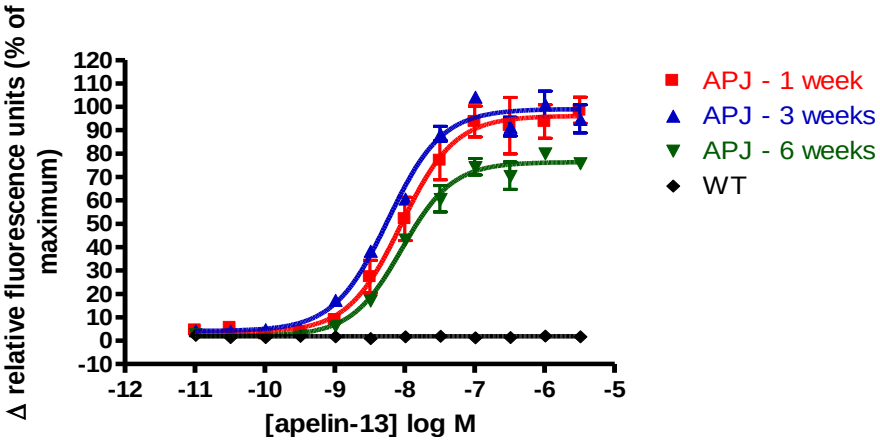

Figure 1. Representative data for activation of APJ receptor stably expressed in Chem-5 cells induced by Apelin 13 using a fluorescent calcium flux assay. APJ–expressing Chem-5 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a fluorescent 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-5) were tested to determine the specificity of the resulting signal.

Table 1. EC_{50} value of APJ-expressing Chem-5 cells.

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
Apelin 13	Calcium Flux - Fluorescence	100	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. The Z' value, as defined with response to 10µM 2MeSATP, was 0.7.

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
	Hygromycin	500 µg/ml	Invivogen: ant-hg-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
NECA ligand	Sigma: E2387
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.

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HOST CELL

Chem-5, an adherent cell line expressing the promiscuous G-protein, Gα15.

EXOGENOUS GENE EXPRESSION

Human APJ cDNA (Accession Number: U03642; see CODING SEQUENCE below) and promiscuous G protein expressed in a bicistronic vector

CODING SEQUENCE

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   1 - ATG GAG GAA GGT GGT GAT TTT GAC AAC TAC TAT GGG GCA GAC AAC CAG TCT GAG TGT GAG  - 60
   1 -  M   E   E   G   G   D   F   D   N   Y   Y   G   A   D   N   Q   S   E   C   E  - 20

  61 - TAC ACA GAC TGG AAA TCC TCG GGG GCC CTC ATC CCT GCC ATC TAC ATG TTG GTC TTC CTC  - 120
  21 -  Y   T   D   W   K   S   S   G   A   L   I   P   A   I   Y   M   L   V   F   L  - 40

 121 - CTG GGC ACC ACG GGA AAC GGT CTG GTG CTC TGG ACC GTG TTT CGG AGC AGC CGG GAG AAG  - 180
  41 -  L   G   T   T   G   N   G   L   V   L   W   T   V   F   R   S   S   R   E   K  - 60

 181 - AGG CGC TCA GCT GAT ATC TTC ATT GCT AGC CTG GCG GTG GCT GAC CTG ACC TTC GTG GTG  - 240
  61 -  R   R   S   A   D   I   F   I   A   S   L   A   V   A   D   L   T   F   V   V  - 80

 241 - ACG CTG CCC CTG TGG GCT ACC TAC ACG TAC CGG GAC TAT GAC TGG CCC TTT GGG ACC TTC  - 300
  81 -  T   L   P   L   W   A   T   Y   T   Y   R   D   Y   D   W   P   F   G   T   F  - 100

 301 - TTC TGC AAG CTC AGC AGC TAC CTC ATC TTC GTC AAC ATG TAC GCC AGC GTC TTC TGC CTC  - 360
 101 -  F   C   K   L   S   S   Y   L   I   F   V   N   M   Y   A   S   V   F   C   L  - 120

 361 - ACC GGC CTC AGC TTC GAC CGC TAC CTG GCC ATC GTG AGG CCA GTG GCC AAT GCT CGG CTG  - 420
 121 -  T   G   L   S   F   D   R   Y   L   A   I   V   R   P   V   A   N   A   R   L  - 140

 421 - AGG CTG CGG GTC AGC GGG GCC GTG GCC ACG GCA GTT CTT TGG GTG CTG GCC GCC CTC CTG  - 480
 141 -  R   L   R   V   S   G   A   V   A   T   A   V   L   W   V   L   A   A   L   L  - 160

 481 - GCC ATG CCT GTC ATG GTG TTA CGC ACC ACC GGG GAC TTG GAG AAC ACC ACT AAG GTG CAG  - 540
 161 -  A   M   P   V   M   V   L   R   T   T   G   D   L   E   N   T   T   K   V   Q  - 180

 541 - TGC TAC ATG GAC TAC TCC ATG GTG GCC ACT GTG AGC TCA GAG TGG GCC TGG GAG GTG GGC  - 600
 181 -  C   Y   M   D   Y   S   M   V   A   T   V   S   S   E   W   A   W   E   V   G  - 200

 601 - CTT GGG GTC TCG TCC ACC ACC GTG GGC TTT GTG GTG CCC TTC ACC ATC ATG CTG ACC TGT  - 660
 201 -  L   G   V   S   S   T   T   V   G   F   V   V   P   F   T   I   M   L   T   C  - 220

 661 - TAC TTC TTC ATC GCC CAA ACC ATC GCT GGC CAC TTC CGC AAG GAA CGC ATC GAG GGC CTG  - 720
 221 -  Y   F   F   I   A   Q   T   I   A   G   H   F   R   K   E   R   I   E   G   L  - 240

 721 - CGG AAG CGG CGC CGG CTG CTC AGC ATC ATC GTG GTG CTG GTG GTG ACC TTT GCC CTG TGC  - 780
 241 -  R   K   R   R   R   L   L   S   I   I   V   V   L   V   V   T   F   A   L   C  - 260

 781 - TGG ATG CCC TAC CAC CTG GTG AAG ACG CTG TAC ATG CTG GGC AGC CTG CTG CAC TGG CCC  - 840
 261 -  W   M   P   Y   H   L   V   K   T   L   Y   M   L   G   S   L   L   H   W   P  - 280

 841 - TGT GAC TTT GAC CTC TTC CTC ATG AAC ATC TTC CCC TAC TGC ACC TGC ATC AGC TAC GTC  - 900
 281 -  C   D   F   D   L   F   L   M   N   I   F   P   Y   C   T   C   I   S   Y   V  - 300

 901 - AAC AGC TGC CTC AAC CCC TTC CTC TAT GCC TTT TTC GAC CCC CGC TTC CGC CAG GCC TGC  - 960
 301 -  N   S   C   L   N   P   F   L   Y   A   F   F   D   P   R   F   R   Q   A   C  - 320

 961 - ACC TCC ATG CTC TGC TGT GGC CAG AGC AGG TGC GCA GGC ACC TCC CAC AGC AGC AGT GGG  - 1020
 321 -  T   S   M   L   C   C   G   Q   S   R   C   A   G   T   S   H   S   S   S   G  - 340

1021 - GAG AAG TCA GCC AGC TAC TCT TCG GGG CAC AGC CAG GGG CCC GGC CCC AAC ATG GGC AAG  - 1080
 341 -  E   K   S   A   S   Y   S   S   G   H   S   Q   G   P   G   P   N   M   G   K  - 360

1081 - GGT GGA GAA CAG ATG CAC GAG AAA TCC ATC CCC TAC AGC CAG GAG ACC CTT GTG GTT GAC  - 1140
 361 -  G   G   E   Q   M   H   E   K   S   I   P   Y   S   Q   E   T   L   V   V   D  - 380

1141 - TGA
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RELATED PRODUCTS

Product Number	Description
HTSCHEM-5	ChemiScreen™ Chem-5 Parental Cell Line (control cells)
HTS068M	ChemiScreen™ APJ Apelin family receptor stable cell line

REFERENCES

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Hosoya M *et al.* (2000) Molecular and functional characteristics of APJ: Tissue distribution of mRNA and interaction with the endogenous ligand apelin. *J. Biol. Chem.* 275: 21061–21067.

Katugampola SD *et al.* (2001) [^{125}I]-(Pyr^{1})Apelin-13 is a novel radioligand for localizing the APJ orphan receptor in human and rat tissues with evidence for a vasoconstrictor role in man. *Br. J. Pharmacol.* 132: 1255-1260.

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Medhurst AD *et al.* (2003) Pharmacological and immunohistochemical characterization of the APJ receptor and its endogenous ligand apelin. *J. Neurochem.* 84: 1162-1172.

Reaux A *et al.* (2001) Physiological role of a novel neuropeptide, apelin, and its receptor in the rat brain. *J. Neurochem.* 77: 1085-1096.

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