eurofins | Discovery Services

PRODUCT DATASHEET

ChemiScreen™ Y_2 Neuropeptide Y Receptor Stable Cell Line

CATALOG NUMBER: HTS066C

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 NPY family consists of three 36-amino acid peptides, neuropeptide Y (NPY), peptide YY (PYY) and pancreatic polypeptide (PP), which bind to the NPY receptor family of G protein-coupled receptors. Five NPY receptors, Y_1, Y_2, Y_4, Y_5 and Y_6, have been defined at the molecular level, and each signals primarily through $G_{i/o}$. Binding of NPY family peptides to NPY receptors mediates a variety of physiological effects, including promotion of food intake, decreased anxiety, inhibition of neurotransmitter and hormone release, vasoconstriction, and gut motility. Y_2 is primarily expressed in the CNS, and it mediates presynaptic inhibition of neurotransmitter release (Michel *et al*., 1998). Cloned human Y_2 receptor-expressing ChemiScreen™ cells were constructed by stable transfection of Chem-1 cells with Y_2. These stability-tested cells are ready for fluorescence-based assays for agonists, antagonists and modulators at the Y_2 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.

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

Calcium Flux Fluorescence Assay

APPLICATION DATA

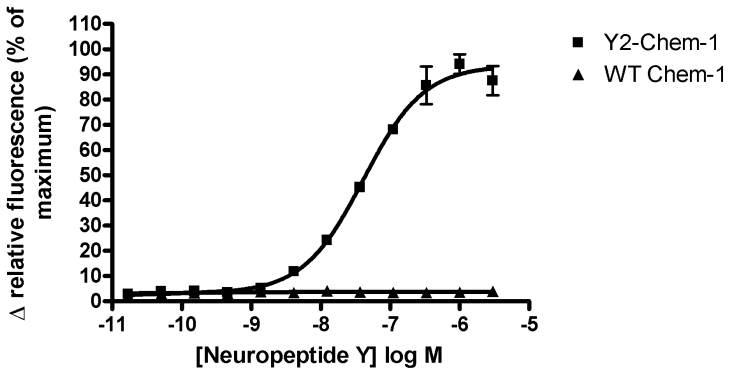

Figure 1. Representative data for activation of Y_2 receptor stably expressed in Chem-1 cells induced by Neuropeptide Y using a fluorescent calcium flux assay. Y_2–expressing Chem-1 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a fluorescent 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 8,000 RLU. Similarly parental cells (catalog #: HTSCHEM-1) were tested to determine the specificity of the resulting signal.

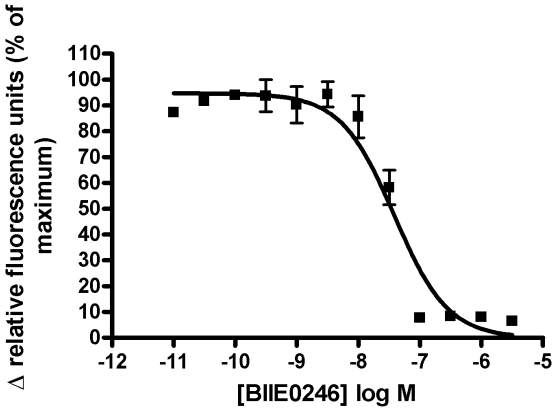

Figure 2. Representative data for inhibition of Y_2 receptor expressed in Chem-1 cells induced by BIIE0246. Y_2–expressing Chem-1 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a fluorescent calcium indicator and preincubated with the indicated concentration of BIIE0246 for 10 min. Calcium flux in response to the 45nM Neuropeptide Y with a final concentration of 0.5% DMSO was determined on a Molecular Devices FLIPR^{TETRA}® with ICCD camera.

Table 1. EC_{50} and IC_{50} value of Y_2-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Neuropeptide Y	Calcium Flux - Fluorescence	45	Eurofins Internal Data
BIIE0246	Calcium Flux - Fluorescence	38	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^{TM}, AM	AAT Bioquest: 21080
Neuropeptide Y ligand	Sigma: N5017
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

NPY2R cDNA (Accession Number: NM_000910; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

```
                                                  ATG GGT CCA ATA GGT GCA GAG GCT GAT GAG AAC
                                                   M   G   P   I   G   A   E   A   D   E   N

CAG ACA GTG GAA GAA ATG AAG GTG GAA CAA TAC GGG CCA CAA ACA ACT CCT AGA GGT GAA CTG GTC CCT
 Q   T   V   E   E   M   K   V   E   Q   Y   G   P   Q   T   T   P   R   G   E   L   V   P

GAC CCT GAG CCA GAG CTT ATA GAT AGT ACC AAG CTG ATT GAG GTA CAA GTT GTT CTC ATA TTG GCC TAC
 D   P   E   P   E   L   I   D   S   T   K   L   I   E   V   Q   V   V   L   I   L   A   Y

TGC TCC ATC ATC TTG CTT GGG GTA ATT GGC AAC TCC TTG GTG ATC CAT GTG GTG ATC AAA TTC AAG AGC
 C   S   I   I   L   L   G   V   I   G   N   S   L   V   I   H   V   V   I   K   F   K   S

ATG CGC ACA GTA ACC AAC TTT TTC ATT GCC AAT CTG GCT GTG GCA GAT CTT TTG GTG AAC ACT CTG TGT
 M   R   T   V   T   N   F   F   I   A   N   L   A   V   A   D   L   L   V   N   T   L   C

CTA CCG TTC ACT CTT ACC TAT ACC TTA ATG GGG GAG TGG AAA ATG GGT CCT GTC CTG TGC CAC CTG GTG
 L   P   F   T   L   T   Y   T   L   M   G   E   W   K   M   G   P   V   L   C   H   L   V

CCC TAT GCC CAG GGC CTG GCA GTA CAA GTA TCC ACA ATC ACC TTG ACA GTA ATT GCC CTG GAC CGG CAC
 P   Y   A   Q   G   L   A   V   Q   V   S   T   I   T   L   T   V   I   A   L   D   R   H

AGG TGC ATC GTC TAC CAC CTA GAG AGC AAG ATC TCC AAG CGA ATC AGC TTC CTG ATT ATT GGC TTG GCC
 R   C   I   V   Y   H   L   E   S   K   I   S   K   R   I   S   F   L   I   I   G   L   A

TGG GGC ATC AGT GCC CTG CTG GCA AGT CCC CTG GCC ATC TTC CGG GAG TAT TCG CTG ATT GAG ATC ATT
 W   G   I   S   A   L   L   A   S   P   L   A   I   F   R   E   Y   S   L   I   E   I   I

CCG GAC TTT GAG ATT GTG GCC TGT ACT GAA AAG TGG CCT GGC GAG GAG AAG AGC ATC TAT GGC ACT GTC
 P   D   F   E   I   V   A   C   T   E   K   W   P   G   E   E   K   S   I   Y   G   T   V

TAT AGT CTT TCT TCC TTG TTG ATC TTG TAT GTT TTG CCT CTG GGC ATT ATA TCA TTT TCC TAC ACT CGC
 Y   S   L   S   S   L   L   I   L   Y   V   L   P   L   G   I   I   S   F   S   Y   T   R

ATT TGG AGT AAA TTG AAG AAC CAT GTC AGT CCT GGA GCT GCA AAT GAC CAC TAC CAT CAG CGA AGG CAA
 I   W   S   K   L   K   N   H   V   S   P   G   A   A   N   D   H   Y   H   Q   R   R   Q

AAA ACC ACC AAA ATG CTG GTG TGT GTG GTG GTG GTG TTT GCG GTC AGC TGG CTG CCT CTC CAT GCC TTC
 K   T   T   K   M   L   V   C   V   V   V   V   F   A   V   S   W   L   P   L   H   A   F

CAG CTT GCC GTT GAC ATT GAC AGC CAG GTC CTG GAC CTG AAG GAG TAC AAA CTC ATC TTC ACA GTG TTC
 Q   L   A   V   D   I   D   S   Q   V   L   D   L   K   E   Y   K   L   I   F   T   V   F

CAC ATT ATC GCC ATG TGC TCC ACT TTT GCC AAT CCC CTT CTC TAT GGC TGG ATG AAC AGC AAC TAC AGA
 H   I   I   A   M   C   S   T   F   A   N   P   L   L   Y   G   W   M   N   S   N   Y   R

AAG GCT TTC CTC TCG GCC TTC CGC TGT GAG CAG CGG TTG GAT GCC ATT CAC TCT GAG GTG TCC GTG ACA
 K   A   F   L   S   A   F   R   C   E   Q   R   L   D   A   I   H   S   E   V   S   V   T

TTC AAG GCT AAA AAG AAC CTG GAG GTC AGA AAG AAC AGT GGC CCC AAT GAC TCT TTC ACA GAG GCT ACC
 F   K   A   K   K   N   L   E   V   R   K   N   S   G   P   N   D   S   F   T   E   A   T

AAT GTC TGA
 N   V  Stp
```

RELATED PRODUCTS

Product Number	Description
HTSCHEM-1	ChemiScreen™ Chem-1 Parental Cell Line (control cells)
HTS066M	ChemiScreen™ Y_2 Neuropeptide Y family receptor membrane prep

REFERENCES

1. Michel MC et al. (1998) XVI. International Union of Pharmacology. Recommendations for the nomenclature of neuropeptide Y, peptide YY and pancreatic polypeptide receptors. Pharmacol. Rev. 50: 143-150.

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.