

## PRODUCT DATASHEET

### ChemiScreen™ GPR41 Free Fatty Acid Receptor Stable Cell Line

#### CATALOG NUMBER: HTS135C

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

**STORAGE:** Vials are to be stored in liquid N<sub>2</sub>.

#### BACKGROUND

ChemiScreen cell lines are constructed in the Chem-4 host, which supports high levels of functional receptor expression on the cell surface. Chem-4 cells contain high endogenous levels of Gα15, a promiscuous G protein, allowing most receptors to couple to the calcium signaling pathway.

GPR41 is a GPCR that, along with GPR43, is activated by short chain carboxylic acids formate, acetate, propionate, butyrate and pentanoate (Brown *et al.*, 2003; Brown *et al.*, 2005). Binding of these ligands to GPR41 selectively activates Gi to inhibit cAMP accumulation. Expression of GPR41 is prominent in adipose tissue, increases during differentiation of cultured adipocytes, and allows short chain carboxylic acids to stimulate leptin synthesis in adipocytes (Xiong *et al.*, 2003). The cloned human GPR41-expressing cell line is made in the Chem-4 host, which supports high levels of recombinant GPR41 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 GPR41 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.

## APPLICATIONS

Calcium Flux Fluorescence Assay

### APPLICATION DATA

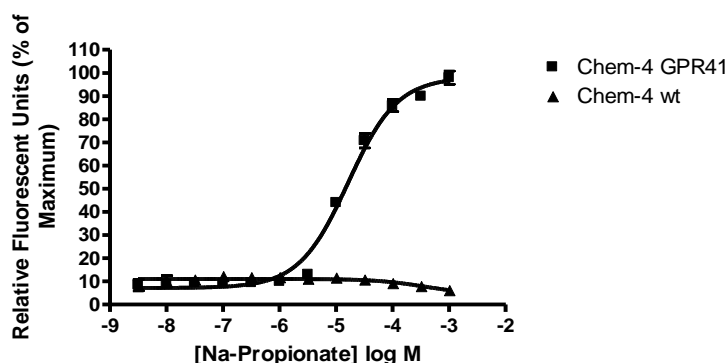


Figure 1. Representative data for activation of the GPR41 receptor stably expressed in Chem-4 cells induced by Sodium Propionate using a fluorescent calcium flux assay. GPR41-expressing Chem-4 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded 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<sup>TETRA</sup>® with ICCD camera. Maximal fluorescence signal obtained in this experiment was 8,000 RLU. Similarly parental cells (catalog #: HTSCHEM-4) were tested to determine the specificity of the resulting signal.

Table 1. EC<sub>50</sub> value of GPR41-expressing Chem-4 cells.

LIGAND	ASSAY	POTENCY EC <sub>50</sub> (nM)	REFERENCE
Sodium Propionate	Calcium Flux - Fluorescence	16	Eurofins Internal Data

\* The cell line was tested and found to have equivalent EC<sub>50</sub> 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)	500 µg/ml	Invivogen: ant-gn-5
Dissociation	Hygromycin	250 µg/ml	Invivogen: ant-hg-5
	Sterile PBS	-	Hyclone: SH30028.03
CryoMedium	0.25% Trypsin-EDTA	-	Hyclone: SH30042.01
	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 37°C Water Bath. Thaw cells rapidly by removing from liquid nitrogen, and immediately immersing in a 37°C 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, 37°C, 5% CO<sub>2</sub>.
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 37° 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 <sup>2</sup> )	Volume (mL)	Total Cell Number (x10 <sup>6</sup> )	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<sup>TETRA</sup>® 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
Sodium Propionate ligand	Sigma: P1880
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  $5 \times 10^5$  cells/ml (i.e, if collected  $5 \times 10^6$  TC,  $\frac{5 \times 10^6}{5 \times 10^5/ml} = 10$  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 37°C 5% CO<sub>2</sub> 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<sup>TETRA</sup>® 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-4, an adherent cell line expressing the promiscuous G-protein, Gα15.

## EXOGENOUS GENE EXPRESSION

Human GPR41 cDNA (Accession Number: NM\_005304; see CODING SEQUENCE below) and promiscuous G protein are expressed in a bicistronic vector

## CODING SEQUENCE

```

                ATG GAT ACA GGC CCC GAC CAG TCC TAC TTC TCC GGC AAT CAC TGG TTC GTC TTC
                M   D   T   G   P   D   Q   S   Y   F   S   G   N   H   W   F   V   F

TCG GTG TAC CTT CTC ACT TTC CTG GTG GGG CTC CCC CTC AAC CTG CTG GCC CTG GTG GTC TTC GTG GGC
S   V   Y   L   L   T   F   L   V   G   L   P   L   N   L   L   A   L   V   V   F   V   G

AAG CTG CAG CGC CGC CCG GTG GCC GTG GAC GTG CTC CTG CTC AAC CTG ACC GCC TCG GAC CTG CTC CTG
K   L   Q   R   R   P   V   A   V   D   V   L   L   L   N   L   T   A   S   D   L   L   L

CTG CTG TTC CTG CCT TTC CGC ATG GTG GAG GCA GCC AAT GGC ATG CAC TGG CCC CTG CCC TTC ATC CTC
L   L   F   L   P   F   R   M   V   E   A   A   N   G   M   H   W   P   L   P   F   I   L

TGC CCA CTC TCT GGA TTC ATC TTC TTC ACC ACC ATC TAT CTC ACC GCC CTC TTC CTG GCA GCT GTG AGC
C   P   L   S   G   F   I   F   F   T   T   I   Y   L   T   A   L   F   L   A   A   V   S

ATT GAA CGC TTC CTG AGT GTG GCC CAC CCA CTG TGG TAC AAG ACC CGG CCG AGG CTG GGG CAG GCA GGT
I   E   R   F   L   S   V   A   H   P   L   W   Y   K   T   R   P   R   L   G   Q   A   G

CTG GTG AGT GTG GCC TGC TGG CTG TTG GCC TCT GCT CAC TGC AGC GTG GTC TAC GTC ATA GAA TTC TCA
L   V   S   V   A   C   W   L   L   A   S   A   H   C   S   V   V   Y   V   I   E   F   S

GGG GAC ATC TCC CAC AGC CAG GGC ACC AAT GGG ACC TGC TAC CTG GAG TTC CGG AAG GAC CAG CTA GCC
G   D   I   S   H   S   Q   G   T   N   G   T   C   Y   L   E   F   R   K   D   Q   L   A

ATC CTC CTG CCC GTG CGG CTG GAG ATG GCT GTG GTC CTC TTT GTG GTC CCG CTG ATC ATC ACC AGC TAC
I   L   L   P   V   R   L   E   M   A   V   V   L   F   V   V   P   L   I   I   T   S   Y

TGC TAC AGC CGC CTG GTG TGG ATC CTC GGC AGA GGG GGC AGC CAC CGC CGG CAG AGG AGG GTG GCG GGC
C   Y   S   R   L   V   W   I   L   G   R   G   G   S   H   R   R   Q   R   R   V   A   G

CTG TTG GCG GCC ACG CTG CTC AAC TTC CTT GTC TGC TTT GGG CCC TAC AAC GTG TCC CAT GTC GTG GGC
L   L   A   A   T   L   L   N   F   L   V   C   F   G   P   Y   N   V   S   H   V   V   G

TAT ATC TGC GGT GAA AGC CCG GCG TGG AGG ATC TAC GTG ACG CTT CTC AGC ACC CTG AAC TCC TGT GTC
Y   I   C   G   E   S   P   A   W   G   I   Y   V   T   L   L   S   T   L   N   S   C   V

GAC CCC TTT GTC TAC TAC TTC TCC TCC TCC GGG TTC CAA GCC GAC TTT CAT GAG CTG CTG AGG AGG TTG
D   P   F   V   Y   Y   F   S   S   S   G   F   Q   A   D   F   H   E   L   L   R   R   L

TGT GGG CTC TGG GGC CAG TGG CAG CAG GAG AGC AGC ATG GAG CTG AAG GAG CAG AAG GGA GGG GAG GAG
C   G   L   W   G   Q   W   Q   Q   E   S   S   M   E   L   K   E   Q   K   G   G   E   E

CAG AGA GCG GAC CGA CCA GCT GAA AGA AAG ACC AGT GAA CAC TCA CAG GGC TGT GGA ACT GGT GGC CAG
Q   R   A   D   R   P   A   E   R   K   T   S   E   H   S   Q   G   C   G   T   G   G   G   G   Q

GTG GCC TGT GCT GAA AGC TGA
V   A   C   A   E   S   Stp

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## RELATED PRODUCTS

Product Number	Description
HTSCHEM-4	ChemiScreen™ Chem-4 Parental Cell Line (control cells)
HTS135M	ChemiScreen™ GPR41 Free Fatty Acid Receptor Membrane Prep

## REFERENCES

1. Brown AJ *et al.* (2003) The orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. *J. Biol. Chem.* 278: 11312-11319.
2. Brown AJ *et al.* (2005) A family of fatty acid binding receptors. *DNA Cell Biol.* 24: 54-61.
3. Xiong Y *et al.* (2004) Short-chain fatty acids stimulate leptin production in adipocytes through the G protein-coupled receptor GPR41. *Proc. Natl. Acad. Sci. USA* 101: 1045-1050.

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