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PRODUCT DATASHEET

ChemiScreen™ GPR109A Nicotinic Acid Receptor Stable Cell Line

CATALOG NUMBER: HTS201C

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-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.

Nicotinic acid (niacin), a vitamin of the B complex, is used clinically in high doses to decrease total plasma levels of cholesterol. Interestingly, the total cholesterol levels and low-density lipoprotein (LDL) concentrations decrease, while the high-density lipoprotein (HDL) concentrations increase with nicotinic acid treatment. The cholesterol-lowering effects of nicotinic acid result from inhibition of lipolysis in adipose tissue, which decreases plasma levels of free fatty acid (FFA) (Altschul *et al.*, 1955; Carlson, 1963). In a study of nicotinic acid and myocardial infarction in the Coronary Drug Project (1966-1975), patients receiving 3 g/day nicotinic acid exhibited reduced rates of myocardial infarction (Coronary Drug Project Research Group, 1975). However, an unwanted effect of high doses of nicotinic acid is vasodilatation, occurring mainly in the upper body and face, known as flushing. Recently two receptors for nicotinic acid have been identified as class A G protein-coupled receptors that couple to G_i to inhibit accumulation of cAMP (Offermans, 2006). GPR109A (also known as HM74A in humans and PUMA-G in mice) is a high affinity receptor for nicotinic acid, whereas GPR109B (also known as HM74) is a low affinity receptor for nicotinic acid that is found in humans but not rodents (Wise *et al*., 2003). GPCR109A is found primarily in adipose tissue and immune cells. Cloned human GPR109A receptor-expressing ChemiScreen cells were constructed by stable transfection of Chem-4 cells with GPR109A 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 GPR109A receptor.

USE RESTRICTIONS

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WARNINGS

For Research Use Only; Not for Use in Diagnostic Procedures
Not for Animal or Human Consumption

GMO

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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.
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Denna produkt innehåller genetiskt ändrade organismer.

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APPLICATIONS

Calcium Flux Fluorescence Assay

APPLICATION DATA

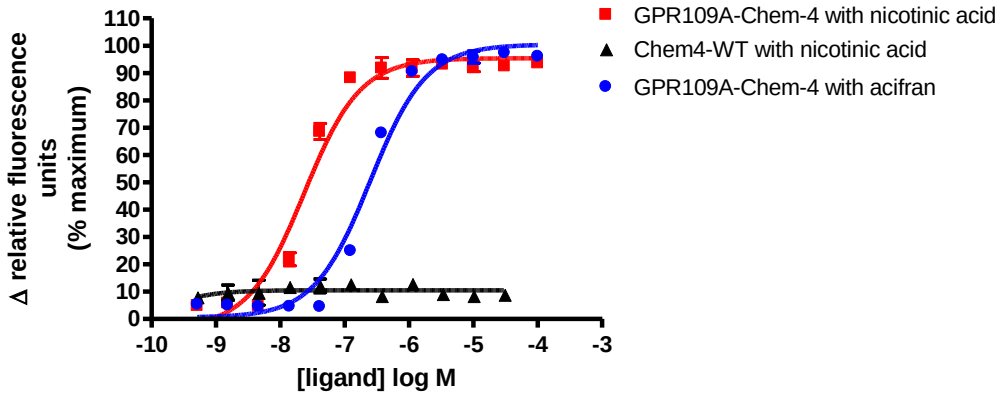

Figure 1. Representative data for activation of GPR109A receptor stably expressed in Chem-4 cells induced by Nicotinic Acid and Acifran using a fluorescent calcium flux assay. GPR109A–expressing Chem-4 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were 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^{TETRA}® with ICCD camera. Maximal fluorescence signal obtained in this experiment was 3,000 RLU. Similarly parental cells were tested to determine the specificity of the resulting signal.

Table 1. EC_{50} values of GPR109A-expressing Chem-4 cells.

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
Nicotinic Acid	Calcium Flux - Fluorescence	18.8	Eurofins Internal Data
Acifran	Calcium Flux - Fluorescence	249	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
	Hygromycin	250 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
Nicotinic Acid	Sigma: N4126
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-4, an adherent rat hematopoietic cell line expressing endogenous G□15 protein as well as an exogenous proprietary promiscuous Gα protein.

EXOGENOUS GENE EXPRESSION

GPR109A cDNA (Accession Number: NM_177551.3; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE:

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   1 - ATG AAT CGG CAC CAT CTG CAG GAT CAC TTT CTG GAA ATA GAC AAG AAG AAC TGC TGT GTG TTC CGA GAT  - 69
   1 -  M   N   R   H   H   L   Q   D   H   F   L   E   I   D   K   K   N   C   C   V   F   R   D  - 23

  70 - GAC TTC ATT GTC AAG GTG TTG CCG CCG GTG TTG GGG CTG GAG TTT ATC TTC GGG CTT CTG GGC AAT GGC  - 138
  24 -  D   F   I   V   K   V   L   P   P   V   L   G   L   E   F   I   F   G   L   L   G   N   G  - 46

 139 - CTT GCC CTG TGG ATT TTC TGT TTC CAC CTC AAG TCC TGG AAA TCC AGC CGG ATT TTC CTG TTC AAC CTG  - 207
  47 -  L   A   L   W   I   F   C   F   H   L   K   S   W   K   S   S   R   I   F   L   F   N   L  - 69

 208 - GCA GTG GCT GAC TTT CTA CTG ATC ATC TGC CTG CCC TTC CTG ATG GAC AAC TAT GTG AGG CGT TGG GAC  - 276
  70 -  A   V   A   D   F   L   L   I   I   C   L   P   F   L   M   D   N   Y   V   R   R   W   D  - 92

 277 - TGG AAG TTT GGG GAC ATC CCT TGC CGG CTG ATG CTC TTC ATG TTG GCT ATG AAC CGC CAG GGC AGC ATC  - 345
  93 -  W   K   F   G   D   I   P   C   R   L   M   L   F   M   L   A   M   N   R   Q   G   S   I  - 115

 346 - ATC TTC CTC ACG GTG GTG GCG GTA GAC AGG TAT TTC CGG GTG GTC CAT CCC CAC CAC GCC CTG AAC AAG  - 414
 116 -  I   F   L   T   V   V   A   V   D   R   Y   F   R   V   V   H   P   H   H   A   L   N   K  - 138

 415 - ATC TCC AAT CGG ACA GCA GCC ATC ATC TCT TGC CTT CTG TGG GGC ATC ACT ATT GGC CTG ACA GTC CAC  - 483
 139 -  I   S   N   R   T   A   A   I   I   S   C   L   L   W   G   I   T   I   G   L   T   V   H  - 161

 484 - CTC CTG AAG AAG AAG ATG CCG ATC CAG AAT GGC GGT GCA AAT TTG TGC AGC AGC TTC AGC ATC TGC CAT  - 552
 162 -  L   L   K   K   K   M   P   I   Q   N   G   G   A   N   L   C   S   S   F   S   I   C   H  - 184

 553 - ACC TTC CAG TGG CAC GAA GCC ATG TTC CTC CTG GAG TTC TTC CTG CCC CTG GGC ATC ATC CTG TTC TGC  - 621
 185 -  T   F   Q   W   H   E   A   M   F   L   L   E   F   F   L   P   L   G   I   I   L   F   C  - 207

 622 - TCA GCC AGA ATT ATC TGG AGC CTG CGG CAG AGA CAA ATG GAC CGG CAT GCC AAG ATC AAG AGA GCC ATC  - 690
 208 -  S   A   R   I   I   W   S   L   R   Q   R   Q   M   D   R   H   A   K   I   K   R   A   I  - 230

 691 - ACC TTC ATC ATG GTG GTG GCC ATC GTC TTT GTC ATC TGC TTC CTT CCC AGC GTG GTT GTG CGG ATC CGC  - 759
 231 -  T   F   I   M   V   V   A   I   V   F   V   I   C   F   L   P   S   V   V   V   R   I   R  - 253

 760 - ATC TTC TGG CTC CTG CAC ACT TCG GGC ACG CAG AAT TGT GAA GTG TAC CGC TCG GTG GAC CTG GCG TTC  - 828
 254 -  I   F   W   L   L   H   T   S   G   T   Q   N   C   E   V   Y   R   S   V   D   L   A   F  - 276

 829 - TTT ATC ACT CTC AGC TTC ACC TAC ATG AAC AGC ATG CTG GAC CCC GTG GTG TAC TAC TTC TCC AGC CCA  - 897
 277 -  F   I   T   L   S   F   T   Y   M   N   S   M   L   D   P   V   V   Y   Y   F   S   S   P  - 299

 898 - TCC TTT CCC AAC TTC TTC TCC ACT TTG ATC AAC CGC TGC CTC CAG AGG AAG ATG ACA GGT GAG CCA GAT  - 966
 300 -  S   F   P   N   F   F   S   T   L   I   N   R   C   L   Q   R   K   M   T   G   E   P   D  - 322

 967 - AAT AAC CGC AGC ACG AGC GTC GAG CTC ACA GGG GAC CCC AAC AAA ACC AGA GGC GCT CCA GAG GCG TTA  - 1035
 323 -  N   N   R   S   T   S   V   E   L   T   G   D   P   N   K   T   R   G   A   P   E   A   L  - 345

1036 - ATG GCC AAC TCC GGT GAG CCA TGG AGC CCC TCT TAT CTG GGC CCA ACC TCT CCT TAA
 346 -  M   A   N   S   G   E   P   W   S   P   S   Y   L   G   P   T   S   P  Stp
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RELATED PRODUCTS

Product Number	Description
HTS201M	ChemiScreen™ GPR109A Nicotinic Acid family receptor membrane prep

REFERENCES

1. Altschul R et al. (1955) Influence of nicotinic acid on serum cholesterol in man. Arch. Biochem. 54,558-559.
2. Carlson LA (1963) Studies on the effect of nicotinic acid on catecholamine stimulated lipolysis in adipose tissue in vitro. Acta Med. Scand. 173: 719-722.
3. Coronary Drug Project Research Group (1975) Clofibrate and niacin in coronary heart disease. J. Am. Med. Assoc. 231: 360-381.
4. Offermans S (2006) The nicotinic acid receptor GPR109A (HM74A or PUMA-G) as a new therapeutic target. Trends Pharmacol. Sci. 27: 384-390.
5. Wise A et al. (2003) Molecular identification of high and low affinity receptors for nicotinic acid. J. Biol. Chem.278: 9869-9874.

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