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PRODUCT DATASHEET

Ready-to-Assay™ GPR109A Nicotinic Acid Receptor Frozen Cells

CATALOG NUMBER: HTS201RTA

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.

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 Gi 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-expressing cell line is made in the Chem-4 host, which supports high levels of recombinant GPR109A expression on the cell surface and contains optimized levels of a recombinant promiscuous G protein to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists, antagonists, and modulators at GPR109A.

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

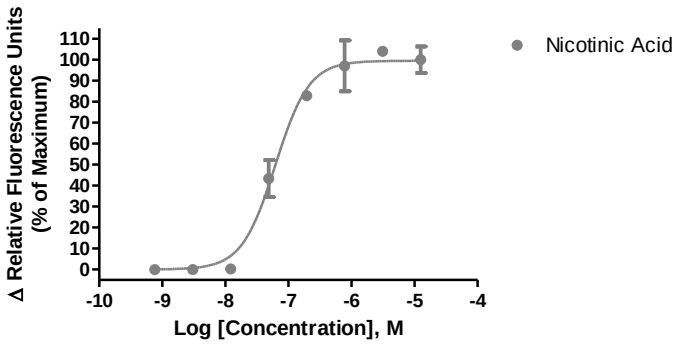

Figure 1. Representative data for activation of GPR109A receptor. Calcium flux in GPR109A –expressing Chem-4 cell line induced by Nicotinic Acid. GPR109A –expressing Chem-4 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA} with ICCD camera. Maximal fluorescence signal obtained in this experiment was 7,500 RLU (Relative Light Units).

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

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Nicotinic Acid	Calcium Flux	60	Eurofins Internal Data
Acifran (Data not shown)	Calcium Flux	250	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
Nicotinic Acid ligand	Sigma: N4126
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-4, an adherent rat hematopoietic cell line expressing endogenous Gα15 protein as well as an exogenous proprietary promiscuous Gα protein.

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EXONGENOUS 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
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS201M	ChemiScreen™ GPR109A Nicotinic Acid receptor membrane prep

Note: Chem-4 cells are derived from Chem-1 cells.

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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