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PRODUCT DATASHEET

Ready-to-Assay™ SUCNR1 / GPR91 Succinate Receptor Frozen Cells

CATALOG NUMBER: HTS241RTA

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.

SUCNR1, or GPR91, is a class A G-protein coupled receptor that binds selectively to succinate, an intermediate in the citric acid cycle, and couples to both the G_i/G_o and G_q pathways. Renal proximal tubules are a major site of GPR91 expression, and succinate-mediated activation of renal GPR91 stimulates the release of renin to cause vasoconstriction and hypertension (He *et al*., 2004). In addition, GPR91 in retinal neurons mediates retinal vascularization induced by succinate, which accumulates in response to hypoxia (Sapieha *et al.,* 2008). Dendritic cells also highly express GPR91, which mediates succinate-induced immune cell migration and activation *in vitro* and *in vivo* (Rubic *et al*., 2008). Therefore, suppression of GPR91 is a potential strategy for treatment of vascular retinopathies, hypertension, and inflammation. Cloned human GPR91 -expressing cell line is made in the Chem-1 host, which supports high levels of recombinant GPR91 expression on the cell surface and contains high levels of the promiscuous G protein to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists and antagonists at GPR91.

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.

Eurofins Pharma Bioanalytics Services US Inc.
15 Research Park Drive
St Charles MO 63304
USA
T | +1 844 522 7787
F | +1 636 362 7131
www.eurofins.com

APPLICATIONS

Calcium Flux Assays

APPLICATION DATA

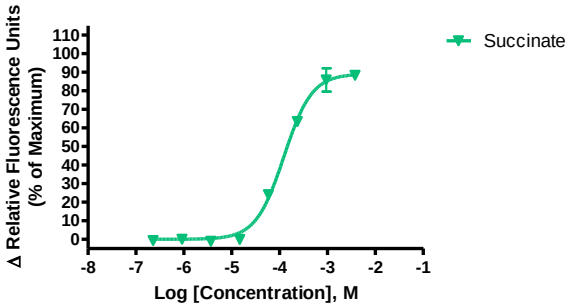

Figure 1. Representative data for activation of GPR91 Succinate receptor. Calcium flux in GPR91–expressing Chem-1 cell line induced by Succinate. GPR91–expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), was determined utilizing 4-fold serial dilution series with each concentration performed in duplicate on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 4,500 RLU (Relative Light Units).

Table 1. EC_{50} value of GPR91-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (µM)	REFERENCE
Succinate	Calcium Flux	120	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
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-1, an adherent rat hematopoietic cell line expressing endogenous Gα15 protein.

EXONGENOUS GENE EXPRESSION

GPR91 cDNA (Accession Number: NM_033050; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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ATG CTG GGG ATC ATG GCA TGG AAT GCA ACT TGC AAA AAC TGG CTG GCA GCA GAG GCT GCC CTG GAA AAG
 M   L   G   I   M   A   W   N   A   T   C   K   N   W   L   A   A   E   A   A   L   E   K

TAC TAC CTT TCC ATT TTT TAT GGG ATT GAG TTC GTT GTG GGA GTC CTT GGA AAT ACC ATT GTT GTT TAC
 Y   Y   L   S   I   F   Y   G   I   E   F   V   V   G   V   L   G   N   T   I   V   V   Y

GGC TAC ATC TTC TCT CTG AAG AAC TGG AAC AGC AGT AAT ATT TAT CTC TTT AAC CTC TCT GTC TCT GAC
 G   Y   I   F   S   L   K   N   W   N   S   S   N   I   Y   L   F   N   L   S   V   S   D

TTA GCT TTT CTG TGC ACC CTC CCC ATG CTG ATA AGG AGT TAT GCC AAT GGA AAC TGG ATA TAT GGA GAC
 L   A   F   L   C   T   L   P   M   L   I   R   S   Y   A   N   G   N   W   I   Y   G   D

GTG CTC TGC ATA AGC AAC CGA TAT GTG CTT CAT GCC AAC CTC TAT ACC AGC ATT CTC TTT CTC ACT TTT
 V   L   C   I   S   N   R   Y   V   L   H   A   N   L   Y   T   S   I   L   F   L   T   F

ATC AGC ATA GAT CGA TAC TTG ATA ATT AAG TAT CCT TTC CGA GAA CAC CTT CTG CAA AAG AAA GAG TTT
 I   S   I   D   R   Y   L   I   I   K   Y   P   F   R   E   H   L   L   Q   K   K   E   F

GCT ATT TTA ATC TCC TTG GCC ATT TGG GTT TTA GTA ACC TTA GAG TTA CTA CCC ATA CTT CCC CTT ATA
A   I   L   I   S   L   A   I   W   V   L   V   T   L   E   L   L   P   I   L   P   L   I

AAT CCT GTT ATA ACT GAC AAT GGC ACC ACC TGT AAT GAT TTT GCA AGT TCT GGA GAC CCC AAC TAC AAC
 N   P   V   I   T   D   N   G   T   T   C   N   D   F   A   S   S   G   D   P   N   Y   N

CTC ATT TAC AGC ATG TGT CTA ACA CTG TTG GGG TTC CTT ATT CCT CTT TTT GTG ATG TGT TTC TTT TAT
 L   I   Y   S   M   C   L   T   L   L   G   F   L   I   P   L   F   V   M   C   F   F   Y

TAC AAG ATT GCT CTC TTC CTA AAG CAG AGG AAT AGG CAG GTT GCT ACT GCT CTG CCC CTT GAA AAG CCT
 Y   K   I   A   L   F   L   K   Q   R   N   R   Q   V   A   T   A   L   P   L   E   K   P

CTC AAC TTG GTC ATC ATG GCA GTG GTA ATC TTC TCT GTG CTT TTT ACA CCC TAT CAC GTC ATG CGG AAT
 L   N   L   V   I   M   A   V   V   I   F   S   V   L   F   T   P   Y   H   V   M   R   N

GTG AGG ATC GCT TCA CGC CTG GGG AGT TGG AAG CAG TAT CAG TGC ACT CAG GTC GTC ATC AAC TCC TTT
 V   R   I   A   S   R   L   G   S   W   K   Q   Y   Q   C   T   Q   V   V   I   N   S   F

TAC ATT GTG ACA CGG CCT TTG GCC TTT CTG AAC AGT GTC ATC AAC CCT GTC TTC TAT TTT CTT TTG GGA
 Y   I   V   T   R   P   L   A   F   L   N   S   V   I   N   P   V   F   Y   F   L   L   G

GAT CAC TTC AGG GAC ATG CTG ATG AAT CAA CTG AGA CAC AAC TTC AAA TCC CTT ACA TCC TTT AGC AGA
D   H   F   R   D   M   L   M   N   Q   L   R   H   N   F   K   S   L   T   S   F   S   R

TGG GCT CAT GAA CTC CTA CTT TCA TTC AGA GAA AAG TGA
 W   A   H   E   L   L   L   S   F   R   E   K  Stp
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)

REFERENCES

1. He W *et al.* (2004) Citric acid cycle intermediates as ligands for orphan G-protein-coupled receptors. *Nature.* 429: 188-193.
2. Rubic T et al. (2008) Triggering the succinate receptor GPR91 on dendritic cells enhances immunity. Nat. Immunol. 9: 1261-1269.
3. Sapieha P *et al.* (2008) The succinate receptor GPR91 in neurons has a major role in retinal angiogenesis. *Nat. Med.* 14: 1067-1076.

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