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PRODUCT DATASHEET

Ready-to-Assay™ GAL_1 Galanin Receptor Frozen Cells

CATALOG NUMBER: HTS094RTA

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.

Galanin is a 29-30 amino acid peptide originally purified from intestine but later found to be abundant in the CNS. A family of 3 GPCRs, GAL_1, GAL_2 and GAL_3, bind to galanin and mediate its biological effects. GAL_1 couples to Gi/o to decrease intracellular cAMP levels (*Wang et al., 1998*). Galanin and its receptors have been implicated in pain, cognition, seizure activity, depression and drug addiction (*Hökfelt, 2005*). In particular, GAL_1 mediates the anticonvulsant activity of galanin. Mice lacking GAL1 are affected by spontaneous seizures, and have increased susceptibility to seizures induced by lithium-pilocarpine treatment and electrical perforant path stimulation (*McColl et al.*, *2006; Mazarati et al., 2004*). Cloned human GAL_1-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant GAL_1 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 agonists, antagonists and modulators at GAL_1.

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

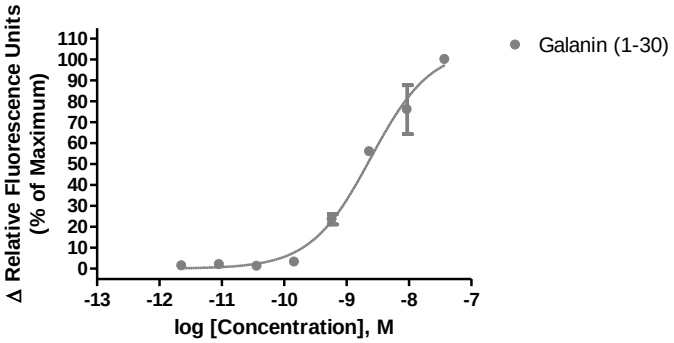

Figure 1. Representative data for activation of GAL_1 receptor. Calcium flux in GAL_1–expressing Chem-1 cell line induced by Galanin (1-30). GAL_1–expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand, 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 1,000 RLU (Relative Light Units).

Table 1. EC_{50} value of GAL_1-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Galanin (1-30)	Calcium Flux	3	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
Galanin (1-30) ligand	Tocris: 1179
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

GALR1 cDNA (Accession Number: NM_001480; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

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CODING SEQUENCE

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                                                ATG GAG CTG GCG GTC GGG AAC CTC AGC GAG GGC AAC
                                                 M   E   L   A   V   G   N   L   S   E   G   N

GCG AGC TGG CCG GAG CCC CCC GCC CCG GAG CCC GGG CCG CTG TTC GGC ATC GGC GTG GAG AAC TTC GTC
 A   S   W   P   E   P   P   A   P   E   P   G   P   L   F   G   I   G   V   E   N   F   V

ACG CTG GTG GTG TTC GGC CTG ATC TTC GCG CTG GGT GTG CTG GGC AAC AGC CTA GTG ATC ACC GTG CTG
 T   L   V   V   F   G   L   I   F   A   L   G   V   L   G   N   S   L   V   I   T   V   L

GCG CGC AGC AAG CCG GGC AAG CCG CGG AGC ACC ACC AAC CTG TTC ATC CTC AAC CTG AGC ATC GCC GAC
 A   R   S   K   P   G   K   P   R   S   T   T   N   L   F   I   L   N   L   S   I   A   D

CTG GCC TAC CTG CTC TTC TGC ATC CCC TTC CAG GCC ACC GTG TAC GCG CTG CCC ACC TGG GTG CTG GGC
 L   A   Y   L   L   F   C   I   P   F   Q   A   T   V   Y   A   L   P   T   W   V   L   G

GCC TTC ATC TGC AAG TTC ATC CAC TAC TTC TTC ACC GTG TCC ATG CTG GTG AGC ATC TTC ACC CTG GCC
 A   F   I   C   K   F   I   H   Y   F   F   T   V   S   M   L   V   S   I   F   T   L   A

GCG ATG TCC GTG GAC CGC TAC GTG GCC ATC GTG CAC TCG CGG CGC TCC TCC TCC CTC AGG GTG TCC CGC
 A   M   S   V   D   R   Y   V   A   I   V   H   S   R   R   S   S   S   L   R   V   S   R

AAC GCG CTG CTG GGC GTG GGC TGC ATC TGG GCG CTG TCC ATT GCC ATG GCC TCG CCC GTG GCC TAC CAC
 N   A   L   L   G   V   G   C   I   W   A   L   S   I   A   M   A   S   P   V   A   Y   H

CAG GGC CTC TTC CAC CCG CGC GCC AGC AAC CAG ACC TTC TGC TGG GAG CAG TGG CCC GAC CCT CGC CAC
 Q   G   L   F   H   P   R   A   S   N   Q   T   F   C   W   E   Q   W   P   D   P   R   H

AAG AAG GCC TAC GTG GTG TGC ACC TTC GTC TTC GGC TAC CTG CTG CCG CTC CTG CTC ATC TGC TTC TGC
 K   K   A   Y   V   V   C   T   F   V   F   G   Y   L   L   P   L   L   L   I   C   F   C

TAT GCC AAG GTC CTT AAT CAC TTG CAT AAA AAG TTG AAG AAC ATG TCA AAG AAG TCT GAA GCA TCC AAG
 Y   A   K   V   L   N   H   L   H   K   K   L   K   N   M   S   K   K   S   E   A   S   K

AAA AAG ACT GCA CAG ACA GTT CTG GTG GTG GTT GTG GTG TTT GGA ATC TCC TGG CTG CCG CAC CAC ATC
 K   K   T   A   Q   T   V   L   V   V   V   V   V   F   G   I   S   W   L   P   H   H   I

ATC CAT CTC TGG GCT GAG TTT GGA GTT TTC CCG CTG ACG CCG GCT TCC TTC CTC TTC AGA ATC ACC GCC
 I   H   L   W   A   E   F   G   V   F   P   L   T   P   A   S   F   L   F   R   I   T   A

CAC TGC CTG GCG TAC AGC AAT TCC TCC GTG AAT CCT ATC ATT TAT GCA TTT CTC TCT GAA AAT TTC AGG
 H   C   L   A   Y   S   N   S   S   V   N   P   I   I   Y   A   F   L   S   E   N   F   R

AAG GCC TAT AAA CAA GTG TTC AAG TGT CAC ATT CGC AAA GAT TCA CAC CTG AGT GAT ACT AAA GAA AAT
 K   A   Y   K   Q   V   F   K   C   H   I   R   K   D   S   H   L   S   D   T   K   E   N

AAA AGT CGA ATA GAC ACC CCA CCA TCA ACC AAT TGT ACT CAT GTG TGA
 K   S   R   I   D   T   P   P   S   T   N   C   T   H   V  Stp
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS094M	ChemiScreen™ GAL_1 Galanin Receptor membrane prep

REFERENCES

1. Hökfelt T (2005) Galanin and its receptors: Introduction to the Third International Symposium, San Diego, California, USA, 21-22 October 2004. Neuropeptides 39: 125-142.
2. Mazarati A et al. (2004) Patterns of seizures, hippocampal injury and neurogenesis in three models of status epilepticus in galanin receptor type 1 (GalR1) knockout mice. Neuroscience 128: 431-41.
3. McColl CD et al. (2006) Galanin receptor-1 knockout mice exhibit spontaneous epilepsy, abnormal EEGs and altered inhibition in the hippocampus. Neuropharmacology 50: 209-18.
4. Wang S et al. (1998) Differential intracellular signaling of the GalR1 and GalR2 galanin receptor subtypes. Biochemistry 37: 6711-7.

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