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PRODUCT DATASHEET

Ready-to-Assay™ GAL_2 Galanin Receptor Frozen Cells

CATALOG NUMBER: HTS186RTA

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. It is widely distributed in tissues such as the brain, spinal cord and gut, and can regulate numerous processes including feeding, nociception, nerve regeneration, memory, neuroendocrine release, and gut secretion and contractility. Galanin elicits its physiological effects through the stimulation of at least three G protein-coupled receptors (*Branchek et al. 2000*). GAL_2 receptor couples predominantly to the activation of phospholipase C. It plays an important role in modulating neurite outgrowth and has been demonstrated to be the principal receptor subtype that mediates the protective effects of galanin in the hippocampus (*Elliott-Hunt et al. 2007*). Cloned human GAL_2-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant GAL_2 expression on the cell surface for functional detection via the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists, antagonists and modulators at GAL_2 Receptor.

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

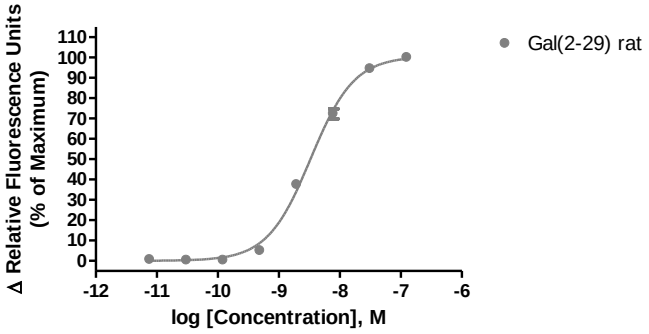

Figure 1. Representative data for activation of GAL_2 receptor. Calcium flux in GAL_2–expressing Chem-1 cell line induced by rat Galanin(2-29). GAL_2–expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand, 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 1,500 RLU (Relative Light Units).

Table 1. EC_{50} values of GAL_2-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Rat Galanin (2-29)	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
Rat Galanin (2-29) ligand	Tocris: 1451
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

GALR2 cDNA (Accession Number: NM_003857; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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                                                ATG AAC GTC TCG GGC TGC CCA GGG GCC GGG AAC GCG
                                                  M   N   V   S   G   C   P   G   A   G   N   A

AGC CAG GCG GGC GGC GGG GGA GGC TGG CAC CCC GAG GCG GTC ATC GTG CCC CTG CTC TTC GCG CTC ATC
 S   Q   A   G   G   G   G   G   W   H   P   E   A   V   I   V   P   L   L   F   A   L   I

TTC CTC GTG GGC ACC GTG GGC AAC ACG CTG GTG CTG GCG GTG CTG CTG CGC GGC GGC CAG GCG GTC AGC
 F   L   V   G   T   V   G   N   T   L   V   L   A   V   L   L   R   G   G   Q   A   V   S

ACT ACC AAC CTG TTC ATC CTT AAC CTG GGC GTG GCC GAC CTG TGT TTC ATC CTG TGC TGC GTG CCC TTC
 T   T   N   L   F   I   L   N   L   G   V   A   D   L   C   F   I   L   C   C   V   P   F

CAG GCC ACC ATC TAC ACC CTG GAC GGC TGG GTG TTC GGC TCG CTG CTG TGC AAG GCG GTG CAC TTC CTC
 Q   A   T   I   Y   T   L   D   G   W   V   F   G   S   L   L   C   K   A   V   H   F   L

ATC TTC CTC ACC ATG CAC GCC AGC AGC TTC ACG CTG GCC GCC GTC TCC CTG GAC AGG TAT CTG GCC ATC
 I   F   L   T   M   H   A   S   S   F   T   L   A   A   V   S   L   D   R   Y   L   A   I

CGC TAC CCG CTG CAC TCC CGC GAG CTG CGC ACG CCT CGA AAC GCG CTG GCA GCC ATC GGG CTC ATC TGG
 R   Y   P   L   H   S   R   E   L   R   T   P   R   N   A   L   A   A   I   G   L   I   W

GGA CTG TCG CTG CTC TTC TCC GGG CCC TAC CTG AGC TAC TAC CGC CAG TCG CAG CTG GCC AAC CTG ACC
 G   L   S   L   L   F   S   G   P   Y   L   S   Y   Y   R   Q   S   Q   L   A   N   L   T

GTG TGC CAT CCC GCG TGG AGC GCC CCT CGC CGC CGC GCC ATG GAC ATC TGC ATC TTC GTC TTC AGC TAC
 V   C   H   P   A   W   S   A   P   R   R   R   A   M   D   I   C   I   F   V   F   S   Y

CTG CTT CCT GTG CTG GTT CTC GGC CTG ACC TAC GCG CGC ACC TTG CGC TAC CTC TGG CGC GCC GTC GAC
 L   L   P   V   L   V   L   G   L   T   Y   A   R   T   L   R   Y   L   W   R   A   V   D

CCG GTG GCC GCG GGC TCG GGT GCC CGG CGC GCC AAG CGC AAG GTG ACA CGC ATG ATC CTC ATC GTG GCC
 P   V   A   A   G   S   G   A   R   R   A   K   R   K   V   T   R   M   I   L   I   V   A

GCG CTC TTC TGC CTC TGC TGG ATG CCC CAC CAC GCG CTC ATC CTC TGC GTG TGG TTC GGC CAG TTC CCG
 A   L   F   C   L   C   W   M   P   H   H   A   L   I   L   C   V   W   F   G   Q   F   P

CTC ACG CGC GCC ACT TAT GCG CTT CGC ATC CTC TCG CAC CTG GTC TCC TAC GCC AAC TCC TGC GTC AAC
 L   T   R   A   T   Y   A   L   R   I   L   S   H   L   V   S   Y   A   N   S   C   V   N

CCC ATC GTT TAC GCG CTG GTC TCC AAG CAC TTC CGC AAA GGC TTC CGC ACG ATC TGC GCG GGC CTG CTG
 P   I   V   Y   A   L   V   S   K   H   F   R   K   G   F   R   T   I   C   A   G   L   L

GGC CGT GCC CCA GGC CGA GCC TCG GGC CGT GTG TGC GCT GCC GCG CGG GGC ACC CAC AGT GGC AGC GTG
 G   R   A   P   G   R   A   S   G   R   V   C   A   A   A   R   G   T   H   S   G   S   V

TTG GAG CGC GAG TCC AGC GAC CTG TTG CAC ATG AGC GAG GCG GCG GGG GCC CTT CGT CCC TGC CCC GGC
 L   E   R   E   S   S   D   L   L   H   M   S   E   A   A   G   A   L   R   P   C   P   G

GCT TCC CAG CCA TGC ATC CTC GAG CCC TGT CCT GGC CCG TCC TGG CAG GGC CCA AAG GCA GGC GAC AGC
 A   S   Q   P   C   I   L   E   P   C   P   G   P   S   W   Q   G   P   K   A   G   D   S

ATC CTG ACG GTT GAT GTG GCC TGA
 I   L   T   V   D   V   A  Stp
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS186M	ChemiScreen™ GAL_2 Galanin Receptor membrane prep

REFERENCES

1. Branchek TA et al. (2000). Galanin receptor subtypes. *Trends Pharmacol. Sci.* 21(3):109-17.
2. Elliott-Hunt CR et al. (2007). Activation of the galanin receptor 2 (GalR2) protects the hippocampus from neuronal damage. *J. Neurochem.* 100(3):780-9.

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