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PRODUCT DATASHEET

ChemiScreen™ GAL_1 Receptor Stable Cell Line

CATALOG NUMBER: HTS094C

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-1 host, which supports high levels of functional receptor expression on the cell surface. Chem-1 cells contain high endogenous levels of Gα15, a promiscuous G protein, allowing most receptors to couple to the calcium signaling pathway.

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 GAL_1 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 antagonists of interactions between GAL_1 and its ligands.

USE RESTRICTIONS

Please see **Limited Use Label License Agreement** (Label License Agreement) for further details.

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 Assay

APPLICATION DATA

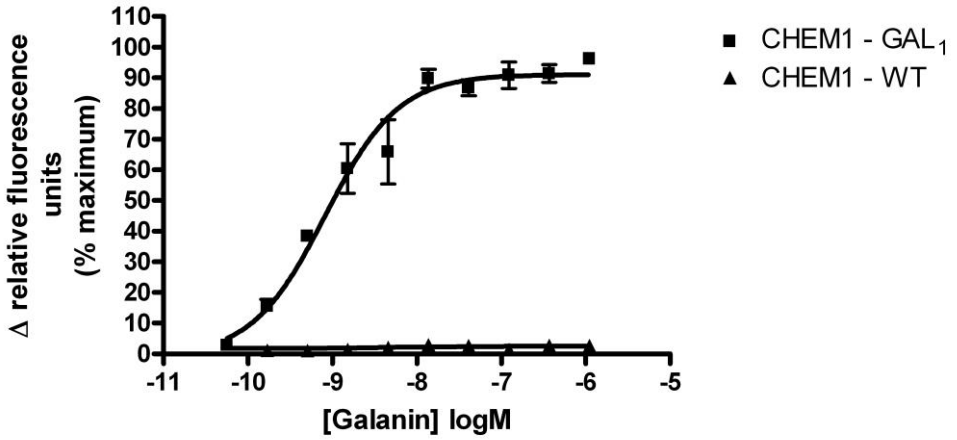

Figure 1. Calcium flux in GAL_1–expressing Chem-1 cell line induced by Galanin. GAL_1–expressing Chem-1 cells and Wild-Type Chem-1 cells (catalog # HTSCHEM-1) were loaded with Fluo-4 NW and calcium flux in response to recombinant human Galanin (10-6 to 10-10.5 M) was determined in triplicate on a Molecular Devices FLIPR TETRA.

Table 1. EC_{50} values of GAL_1 cells.

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
Galanin	Calcium Flux - Fluorescence	1	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
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

HOST CELL

Chem-1, an adherent cell line expressing the promiscuous G-protein, Gα15.

EXOGENOUS GENE EXPRESSION

Full-length human GALR1 cDNA encoding GAL_1 (Accession Number: NM_001480)

RELATED PRODUCTS

Product Number	Description
HTSCHEM-1	ChemiScreen™ Chem-1 Parental Cell Line (control cells)
HTS094RTA	ChemiScreen™ Receptor GAL_1 Ready to Assay Cells

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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