ChemiScreen™ CALCIUM-OPTIMIZED STABLE CELL LINE HUMAN RECOMBINANT GPR91 SUCCINATE RECEPTOR

CATALOG NUMBER:	HTS241C	**QUANTITY:**	2 vials, 1 mL per vial
LOT NUMBER:	RI09010001	**CONCENTRATION:**	2 x 10^6 cells/mL

BACKGROUND:

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. Millipore's 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.

APPLICATIONS:

Calcium flux assay

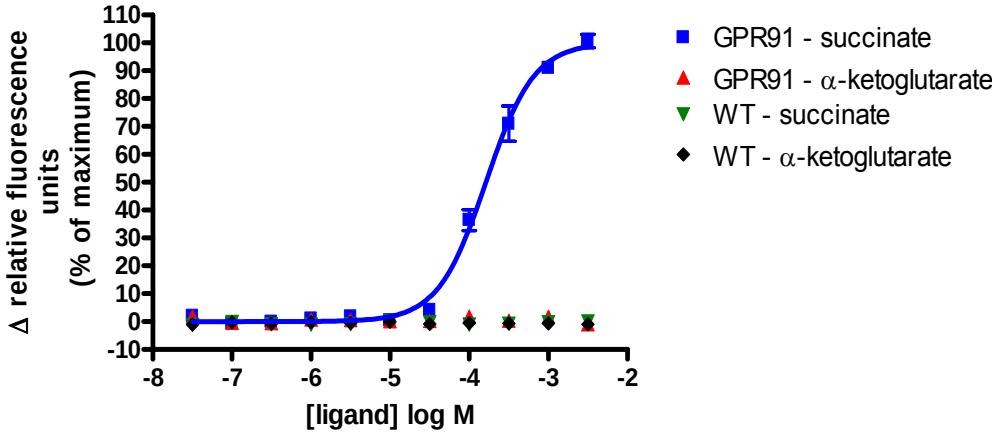

Figure 1. Calcium flux in GPR91-expressing Chem-1 cell line. GPR91-expressing Chem-1 cells and Wild-Type Chem-1 cells (Millipore catalog # HTSCHEM-1) were loaded with a no-wash calcium assay kit, and calcium flux in response to succinate and α-ketoglutarate was determined in triplicate on a Molecular Devices FLIPR$^{TETRA_{TM}}$. In this experiment, average maximum signal was 3500 RLU. Z' was 0.685 with succinate at 2x EC50.

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Table I. Comparison of EC50 values of GPR91-expressing Chem-1 cells with values described in the literature.

ligand	assay	potency (μM)	Reference
succinate	Calcium	EC50 = 143-200	Figure 1
succinate	Calcium	EC50 = 56	He *et al*., 2004

HOST CELLS: Chem-1, a rat adherent cell line of hematopoietic origin expressing the promiscuous G-protein, $G_{\alpha 15}$.

TRANSFECTION: Proprietary plasmid pHS containing GPR91 cDNA (Accession Number: NM_033050; see CODING SEQUENCE below). The stable clonal cell line was selected by resistance to geneticin, followed by limited dilution cloning. The cell line was tested and found to have equivalent EC50 and signal at 1, 3 and 6 weeks of continuous culture.

PRESENTATION:

Cells are frozen at 2 x 10^6 cells/mL in 90% fetal bovine serum/10% DMSO. Cell line tests negative for mycoplasma.

STORAGE/HANDLING:

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. Transfer contents of the vial to a T75 flask containing growth media. Place the flask in a humidified incubator at 37ºC with 5% CO_2.
3. After 8-24 h, all live cells will be attached. Viability of the cells is expected to be 50-80%. At this time, replace media to remove residual DMSO, and return to incubator.
4. When cells are approximately 80% confluent, passage the cells as follows: Remove media and wash once with HBSS without Ca^{++} and Mg^{++} (10 mL/T75). Add 0.05% trypsin/0.2 g/L EDTA (1 mL/T75) and place in humidified incubator at 37ºC with 5% CO_2 until cells begin to round up and detach (5-10 minutes). Gently rap the side of the flask to dislodge the cells. Neutralize trypsin by addition of 4 mL Chem-1 Growth Media per 1 mL trypsin.
5. Cells are typically passaged 1:10 every 3-4 days. Passaging ratio may be varied according to requirements of the investigator.
6. Frozen stocks of cells should be prepared at the earliest passage possible after thawing, as follows: Count detached cells (prepared as in Step 4). Centrifuge cells at 200 x g for 5 min. Resuspend cells at 5 x 10^6 cells/mL in Chem-1 Freezing Media (cell densities of 2-10 x 10^6 are also acceptable if necessary). Dispense 1 mL aliquots into cryopreservation vials. Freeze the cells by a controlled rate process, such as in an isopropanol-jacketed container placed at –70°C overnight. Store the vials in liquid nitrogen.

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7. Use of cells immediately after thawing is feasible for some cell lines and is being further validated. Some cell lines may need to be passaged at least once after thawing prior to use in calcium flux assays. Cells should be resuspended in Chem-1 Plating Media for plating for calcium assay.

MEDIA:

Chem-1 Growth Media:
DMEM with 4.5 g/L glucose and 4 mM glutamine (Millipore SLM-020-A)
10% heat-inactivated FBS
1x Nonessential amino acids (from 100x stock, Millipore TMS-001-C)
10mM HEPES (from 1 M HEPES, Millipore TMS-003-C)
1x Pen-Strep (from 100x stock, Millipore TMS-AB2-C)
250μg/mL Genetecin/G-418
Note: fetal bovine serum contains succinate in varying quantites. Improved succinate-induced calcium signal may be obtained in some cases by propagating and plating GPR91 cells in a serum-free media such as Ultraculture media (Lonza 12-725F)

Chem-1 Plating Media:
DMEM with 4.5 g/L glucose and 4 mM glutamine
10% heat-inactivated FBS
1x NEAA
10mM HEPES
1x Pen-Strep

Chem-1 Freezing Media:
90% heat-inactivated FBS
10% DMSO (cell culture grade)

EXAMPLE ASSAY CONDITIONS:

1. Cells propagated for screening should be maintained and seeded at less than 90% confluency. Trypsinize cells as above and seed cells in 96-well black-walled, clear bottom plate at 50,000 cells/well in Chem-1 Plating Media. Keep the plate at room temperature for 1 h to allow even cell distribution in the plate, then transfer plate to a humidified incubator at 37ºC with 5% CO_2.
2. Chem-1 derived cell lines have been successfully assayed using multiple commercially-available calcium dye kits following the manufacture's protocols. The protocol described below is a suggested protocol that can be generally applied to most calcium dyes kits.
3. Remove media
4. Wash cells with buffered salt solution
5. Add 100 μL/well calcium dye-loading solution.
6. Incubate the plate for 30 minutes in a humidified incubator at 37ºC with 5% CO_2.
7. Incubate the plate for an additional 60 min at 25ºC with 5% CO_2.
8. Set-up FLIPR to dispense 50uL/well 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 at 95 uL and dispense rate

to 25 μL/sec. Set up plate layout and tip layout for each individual experiment. Set time course for 180 seconds, with ligand addition at 10 seconds.

9. Ligands are prepared in a white nonbinding surface 96-well plate (Corning 3605).
10. After the run is complete, negative control correction is applied and data analyzed utilizing the maximum statistic.

REFERENCES:

He W *et al.* (2004) Citric acid cycle intermediates as ligands for orphan G-protein-coupled receptors. *Nature.* 429: 188-193.

Rubic T et al. (2008) Triggering the succinate receptor GPR91 on dendritic cells enhances immunity. Nat. Immunol. 9: 1261-1269.

Sapieha P *et al.* (2008) The succinate receptor GPR91 in neurons has a major role in retinal angiogenesis. *Nat. Med.* 14: 1067-1076.

CODING SEQUENCE:

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 G   Y   I   F   S   L   K   N   W   N   S   S   N   I   Y   L   F   N   L   S   V   S   D

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 V   L   C   I   S   N   R   Y   V   L   H   A   N   L   Y   T   S   I   L   F   L   T   F

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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

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 W   A   H   E   L   L   L   S   F   R   E   K  Stp
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GMO

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Questo prodotto contiene degli organismi geneticamente modificati.
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