ChemiScreen™ CALCIUM-OPTIMIZED STABLE CELL LINE HUMAN RECOMBINANT GPR39 ZINC RECEPTOR

CATALOG NUMBER:	HTS217C	**QUANTITY:**	2 vials, 1 mL per vial
LOT NUMBER:		**CONCENTRATION:**	2 x 10^6 cells/mL

BACKGROUND:

GPR39 is a 7TM protein related to the G protein-coupled receptors for the gastrointestinal peptides ghrelin and motilin. Although GPR39 has been reported to be a receptor for the peptide obestatin, GPR39 has been more thoroughly characterized as a receptor for Zn^{2+}, as well as Cu^{2+} and Ni^{2+} (Holst *et al*., 2007; Yasuda *et al*., 2007). Studies on GPR39-null mice implicate GPR39 in the control of insulin secretion, thereby indicating its potential as a target for treatment of diabetes (Holst *et al*., 2009; Tremblay *et al*., 2009). Millipore's cloned human GPR39 -expressing cell line is made in the HEK293 host, which supports high levels of recombinant GPR39 expression on the cell surface and supports optimal coupling of the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists and antagonists at GPR39.

APPLICATIONS:

Calcium flux assay

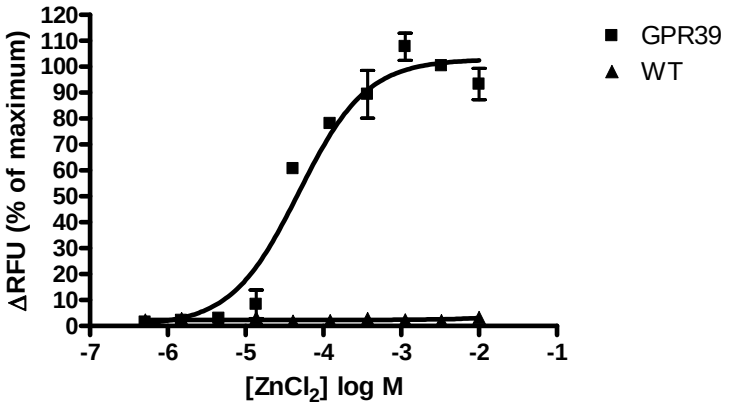

Figure 1. Calcium flux in GPR39-expressing HEK293 cell line. GPR39-expressing HEK293 cells and Wild-Type HEK293 cells were loaded with a no-wash calcium assay kit, and calcium flux in response to $ZnCl_2$ was determined in triplicate on a Molecular Devices FLIPR$^{TETRA_{TM}}$. In this experiment, average maximum signal was 5500 RLU. Z' was 0.64 with $ZnCl_2$ at EC_{80}.

Table I. Comparison of EC50 values of GPR39-expressing HEK293 cells with values described in the literature.

ligand	assay	potency (μM)	Reference
$ZnCl_2$	Calcium	EC50 = 48.4	Figure 1
$ZnCl_2$	Inositol phosphate	EC50 = 22	Holst *et al*., 2007

HOST CELLS: HEK293

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TRANSFECTION: Proprietary plasmid E5 containing GPR39 cDNA (Accession Number: NM_001508; 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 Accutase (Millipore SCR005) at 1 mL/T75 and keep at room temperature until cells begin to round up and detach (5-10 minutes). Gently rap the side of the flask to dislodge the cells. Neutralize Accutase by addition of 4 mL HEK293 Growth Media per 1 mL Accutase.
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 HEK293 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.
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 HEK293 Plating Media for plating for calcium assay.

MEDIA:

HEK293 Growth Media:
DMEM/F12 with 2.5 mM glutamine (Millipore DF-041)
10% heat-inactivated FBS
1x Nonessential amino acids (from 100x stock, Millipore TMS-001-C)
1x Pen-Strep (from 100x stock, Millipore TMS-AB2-C)
250μg/mL Genetecin/G-418

HEK293 Plating Media:
DMEM/F12 with 2.5 mM glutamine
10% heat-inactivated FBS

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1x Nonessential amino acids
1x Pen-Strep

HEK293 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.
2. For seeding cells for assay, use of collagen-coated assay plates is recommended for optimal attachment. The stock collagen solution is 5 mg/mL type I collagen in 2% acetic acid, stored at 4°C. To prepare the assay plates, dilute the stock collagen solution 1:20 in HBSS without Ca^{++} and Mg^{++}, add 200 µL/well in a 96-well black-walled, clear bottom plate, and keep at room temperature for 10 min. Remove the solution and air dry in a sterile laminar flow hood for 15 min.
3. On the day prior to the assay, detach cells with Accutase and neutralize as above. Plate cells at 60,000 cells/well in HEK293 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.
4. HEK293 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.
5. Remove media
6. Wash cells with buffered salt solution
7. Add 100 µL/well calcium dye-loading solution.
8. Incubate the plate for 30 minutes in a humidified incubator at 37ºC with 5% CO_2.
9. Incubate the plate for an additional 60 min at 25ºC with 5% CO_2.
10. 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.
11. Ligands are prepared in a white nonbinding surface 96-well plate (Corning 3605).
12. After the run is complete, negative control correction is applied and data analyzed utilizing the maximum statistic.

REFERENCES:

Holst B. *et al.* (2007) GPR39 signaling is stimulated by zinc ions but not by obestatin. *Endocrinology* 148: 13-20.

Holst B. *et al.* (2009) G protein-coupled receptor 39 deficiency is associated with

pancreatic islet dysfunction. *Endocrinology* 150: 2577-2585.

Tremblay F. et al. (2009) Disruption of G protein-coupled receptor 39 impairs insulin secretion in vivo. *Endocrinology* 150: 2586-2595.

Yasuda S.-I. *et al.* (2007) Isolation of Zn^{2+} as an endogenous agonist of GPR39 from fetal bovine serum. *J. Recept. Signal Transduct. Res.* 27: 235-246.

CODING SEQUENCE:

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   1 - ATG GCT TCA CCC AGC CTC CCG GGC AGT GAC TGC TCC CAA ATC ATT GAT CAC AGT CAT GTC CCC GAG TTT GAG  - 72
   1 -  M   A   S   P   S   L   P   G   S   D   C   S   Q   I   I   D   H   S   H   V   P   E   F   E  - 24

  73 - GTG GCC ACC TGG ATC AAA ATC ACC CTT ATT CTG GTG TAC CTG ATC ATC TTC GTG ATG GGC CTT CTG GGG AAC  - 144
  25 -  V   A   T   W   I   K   I   T   L   I   L   V   Y   L   I   I   F   V   M   G   L   L   G   N  - 48

 145 - AGC GCC ACC ATT CGG GTC ACC CAG GTG CTG CAG AAG AAA GGA TAC TTG CAG AAG GAG GTG ACA GAC CAC ATG  - 216
  49 -  S   A   T   I   R   V   T   Q   V   L   Q   K   K   G   Y   L   Q   K   E   V   T   D   H   M  - 72

 217 - GTG AGT TTG GCT TGC TCG GAC ATC TTG GTG TTC CTC ATC GGC ATG CCC ATG GAG TTC TAC AGC ATC ATC TGG  - 288
  73 -  V   S   L   A   C   S   D   I   L   V   F   L   I   G   M   P   M   E   F   Y   S   I   I   W  - 96

 289 - AAT CCC CTG ACC ACG TCC AGC TAC ACC CTG TCC TGC AAG CTG CAC ACT TTC CTC TTC GAG GCC TGC AGC TAC  - 360
  97 -  N   P   L   T   T   S   S   Y   T   L   S   C   K   L   H   T   F   L   F   E   A   C   S   Y  - 120

 361 - GCT ACG CTG CTG CAC GTG CTG ACA CTC AGC TTT GAG CGC TAC ATC GCC ATC TGT CAC CCC TTC AGG TAC AAG  - 432
 121 -  A   T   L   L   H   V   L   T   L   S   F   E   R   Y   I   A   I   C   H   P   F   R   Y   K  - 144

 433 - GCT GTG TCG GGA CCT TGC CAG GTG AAG CTG CTG ATT GGC TTC GTC TGG GTC ACC TCC GCC CTG GTG GCA CTG  - 504
 145 -  A   V   S   G   P   C   Q   V   K   L   L   I   G   F   V   W   V   T   S   A   L   V   A   L  - 168

 505 - CCC TTG CTG TTT GCC ATG GGT ACT GAG TAC CCC CTG GTG AAC GTG CCC AGC CAC CGG GGT CTC ACT TGC AAC  - 576
 169 -  P   L   L   F   A   M   G   T   E   Y   P   L   V   N   V   P   S   H   R   G   L   T   C   N  - 192

 577 - CGC TCC AGC ACC CGC CAC CAC GAG CAG CCC GAG ACC TCC AAT ATG TCC ATC TGT ACC AAC CTC TCC AGC CGC  - 648
 193 -  R   S   S   T   R   H   H   E   Q   P   E   T   S   N   M   S   I   C   T   N   L   S   S   R  - 216

 649 - TGG ACC GTG TTC CAG TCC AGC ATC TTC GGC GCC TTC GTG GTC TAC CTC GTG GTC CTG CTC TCC GTA GCC TTC  - 720
 217 -  W   T   V   F   Q   S   S   I   F   G   A   F   V   V   Y   L   V   V   L   L   S   V   A   F  - 240

 721 - ATG TGC TGG AAC ATG ATG CAG GTG CTC ATG AAA AGC CAG AAG GGC TCG CTG GCC GGG GGC ACG CGG CCT CCG  - 792
 241 -  M   C   W   N   M   M   Q   V   L   M   K   S   Q   K   G   S   L   A   G   G   T   R   P   P  - 264

 793 - CAG CTG AGG AAG TCC GAG AGC GAA GAG AGC AGG ACC GCC AGG AGG CAG ACC ATC ATC TTC CTG AGG CTG ATT  - 864
 265 -  Q   L   R   K   S   E   S   E   E   S   R   T   A   R   R   Q   T   I   I   F   L   R   L   I  - 288

 865 - GTT GTG ACA TTG GCC GTA TGC TGG ATG CCC AAC CAG ATT CGG AGG ATC ATG GCT GCG GCC AAA CCC AAG CAC  - 936
 289 -  V   V   T   L   A   V   C   W   M   P   N   Q   I   R   R   I   M   A   A   A   K   P   K   H  - 312

 937 - GAC TGG ACG AGG TCC TAC TTC CGG GCG TAC ATG ATC CTC CTC CCC TTC TCG GAG ACG TTT TTC TAC CTC AGC  - 1008
 313 -  D   W   T   R   S   Y   F   R   A   Y   M   I   L   L   P   F   S   E   T   F   F   Y   L   S  - 336

1009 - TCG GTC ATC AAC CCG CTC CTG TAC ACG GTG TCC TCG CAG CAG TTT CGG CGA GTG TTC GTG CAG GTG CTG TGC  - 1080
 337 -  S   V   I   N   P   L   L   Y   T   V   S   S   Q   Q   F   R   R   V   F   V   Q   V   L   C  - 360

1081 - TGC CGC CTG TCG CTG CAG CAC GCC AAC CAC GAG AAG CGC CTG CGC GTA CAT GCG CAC TCC ACC ACC GAC AGC  - 1152
 361 -  C   R   L   S   L   Q   H   A   N   H   E   K   R   L   R   V   H   A   H   S   T   T   D   S  - 384

1153 - GCC CGC TTT GTG CAG CGC CCG TTG CTC TTC GCG TCC CGG CGC CAG TCC TCT GCA AGG AGA ACT GAG AAG ATT  - 1224
 385 -  A   R   F   V   Q   R   P   L   L   F   A   S   R   R   Q   S   S   A   R   R   T   E   K   I  - 408

1225 - TTC TTA AGC ACT TTT CAG AGC GAG GCC GAG CCC CAG TCT AAG TCC CAG TCA TTG AGT CTC GAG TCA CTA GAG  - 1296
 409 -  F   L   S   T   F   Q   S   E   A   E   P   Q   S   K   S   Q   S   L   S   L   E   S   L   E  - 432

1297 - CCC AAC TCA GGC GCG AAA CCA GCC AAT TCT GCT GCA GAG AAT GGT TTT CAG GAG CAT GAA GTT TGA          - 1362
 433 -  P   N   S   G   A   K   P   A   N   S   A   A   E   N   G   F   Q   E   H   E   V  Stp          - 463
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