

**ChemiScreen™ CALCIUM-OPTIMIZED STABLE CELL LINE
HUMAN RECOMBINANT GPR68/OGR1 PROTON-SENSING RECEPTOR**

CATALOG NUMBER:	HTS153C	QUANTITY:	2 vials, 1 mL per vial
LOT NUMBER:		CONCENTRATION:	2 x 10 ⁶ cells/mL

BACKGROUND: GPR68, also known as OGR1 (ovarian cancer G-protein-coupled receptor 1), was initially thought to be a receptor for sphingosylphosphorylcholine, although these results are controversial. Subsequent studies indicated that GPR68/OGR1 functions as a G_q-coupled sensor for extracellular pH, with maximal signaling occurring at pH of 7 or below (Ludwig *et al.*, 2003; Tomura *et al.*, 2005). Expression of GPR68/OGR1 is upregulated during RANKL-induced osteoclast differentiation, and knockdown of GPR68/OGR1 expression inhibits this differentiation process (Yang *et al.*, 2006). Chemicon's cloned human GPR68-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant GPR68 expression on the cell surface and contains high levels of the promiscuous G protein G_α15 to enhance coupling of the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for antagonists of interactions between GPR68 and its ligands.

APPLICATIONS: Calcium flux assay, ligand binding assays

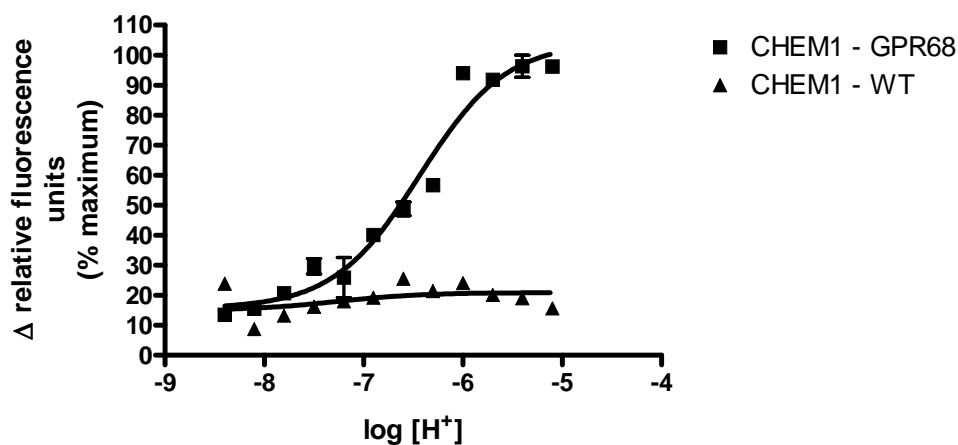


Figure 1. Calcium flux in GPR68-expressing Chem-1 cell line induced by proton concentration. GPR68-expressing Chem-1 cells and Wild-Type Chem-1 cells (Chemicon catalog # HTSCHEM-1) were loaded with Fluo-4 and calcium flux in response to pH (5.1-8.4) was determined in triplicate on a Molecular Devices FLIPR^{TETRA™}.

SPECIFICATIONS: EC50 for calcium mobilization by pH: 6.4

HOST CELLS: Chem-1, an adherent cell line expressing the promiscuous G-protein, G_α15.

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TRANSFECTION: Full-length human GPR68 cDNA encoding OGR₁ (Accession Number: NM_003485)

GROWTH MEDIA: DMEM containing 4.5 g/L glucose/10% heat inactivated fetal bovine serum/1x nonessential amino acids/10 mM HEPES/0.25 mg/ml Geneticin (G418)/100 U/ml each penicillin and streptomycin

- PRESENTATION:** Cells are frozen at 2×10^6 cells/mL in DMEM/20% fetal bovine serum/100 U/ml penicillin and streptomycin/10% DMSO. Cell line tests negative for mycoplasma.
- STORAGE/HANDLING:** Place cells in liquid nitrogen immediately upon receipt. Maintain frozen in liquid nitrogen for up to 5 years. Thaw cells rapidly by removing from liquid nitrogen and immediately immersing in a 37°C 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 20 mL growth media, and place in a humidified 37°C incubator with 5% CO₂. After 8-24 h, cells will adhere to the plate, at which time the media should be replaced to remove residual DMSO. Cells are passaged by washing with Ca⁺⁺ and Mg⁺⁺-free HBSS (10 mL/T75), incubating with 0.05% trypsin/0.2 g/L EDTA (1 mL/T75) for 5-10 minutes at 37°C, and rapping the side of the flask to dislodge the cells. Neutralize the trypsin by addition of 4 volumes growth media. Cells are typically passaged 1:10 with every 3-4 days, and should be passaged at least once after thawing prior to use in calcium flux assays.
- REFERENCES:** Ludwig MG *et al.* (2003) Proton-sensing G-protein coupled receptors. *Nature* 425: 93-98.
- Tomura H *et al.* (2005) Proton-sensing and lysolipid-sensitive G-protein-coupled receptors: A novel type of multi-functional receptors. *Cell Signal*. 17: 1466-1476.
- Yang M *et al.* (2006) Expression of and role for ovarian cancer G-protein-coupled receptor 1 (OGR1) during osteoclastogenesis. *J. Biol. Chem.* 281: 23598-23605.

GMO

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HUMAN RECOMBINANT GPR68/OGR1 PROTON-SENSING RECEPTOR**

Product No. HTS153C

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