

**ChemiScreen™ CALCIUM-OPTIMIZED STABLE CELL LINE
HUMAN RECOMBINANT MrgD Mas-Related RECEPTOR**

CATALOG NUMBER:	HTS206C	QUANTITY:	2 vials, 1 mL per vial
LOT NUMBER:		CONCENTRATION:	2×10^6 cells/mL

BACKGROUND: The Mas-related genes (Mrgs), also known as sensory neuron-specific G-protein coupled receptors (SNSRs), are selectively expressed in subpopulations of sensory neurons involved in the perception of pain (Dong *et al.*, 2001). The Mrg family in rodents consists of 50 receptors in the MrgA-MrgH subfamilies, but humans contain fewer Mrg members. MrgD (also known as TGR7) is found across species from rodents to humans, and its expression is restricted to nonpeptidergic, small-diameter, IB4+ dorsal root ganglion (DRG) neurons. MrgD binds β -alanine, but not L-alanine, which results in increased intracellular calcium and the suppression of forskolin-induced cAMP production, thus suggesting coupling to Gq and Gi proteins (Shinohara *et al.*, 2004). β -alanine-induced activation of MrgD inhibits KCNQ2/3-mediated M-currents in cells overexpressing MrgD and KCNQ2/3 and also in cultured DRG neurons, which indicates that MrgD enhances nociceptive signaling (Crozier *et al.*, 2007). Millipore's cloned human MrgD-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant MrgD expression on the cell surface and contains optimal 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 antagonists of interactions between MrgD and its ligands.

APPLICATIONS: Calcium flux assay, ligand binding assays

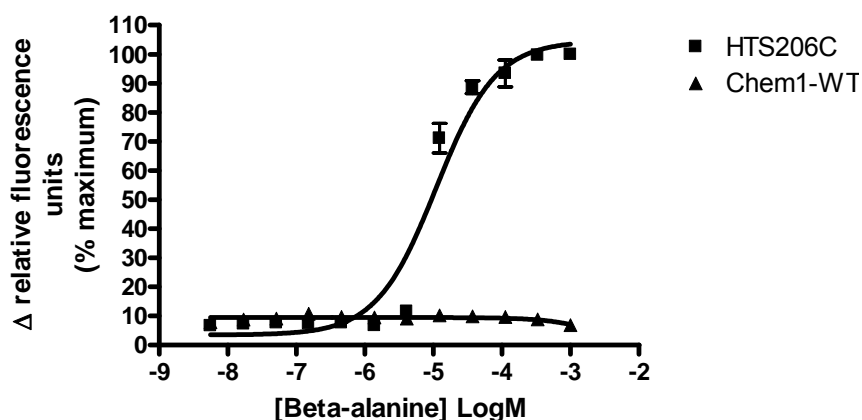


Figure 1. Calcium flux in MrgD-expressing Chem-1 cell line induced by Beta-alanine. MrgD expressing Chem-1 cells and Wild-Type Chem-1 cells were loaded with Fluo-8 NW and calcium flux in response to Beta-alanine (10^{-3} to $10^{-8.25}$ M) was determined in triplicate on a Molecular Devices FLIPR TETRA.

SPECIFICATIONS: EC50 for calcium mobilization by Beta-alanine: $\sim 11.0 \mu\text{M}$

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

TRANSFECTION: Full-length human MRGPRD cDNA (Accession Number: NM_198923;

AY427820)

PRESENTATION:

Cells are frozen at 2×10^6 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. Maintain frozen in liquid nitrogen for up to 5 years.
2. 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 growth media. Place the flask in a humidified incubator at 37°C with 5% CO₂.
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 37°C with 5% CO₂ 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 5×10^6 cells/mL in Chem-1 Freezing Media (cell densities of $2-10 \times 10^6$ 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 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

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)

REFERENCES:

Crozier RA *et al.* (2007). MrgD Activation Inhibits KCNQ/M-Currents and Contributes to Enhanced Neuronal Excitability. *J. Neurosci.* 27(16): 4492-4496.

Dong X *et al.* (2001) A diverse family of GPCRs expressed in specific subsets of nociceptive sensory neurons. *Cell* 106: 619-632.

Shinohara T *et al.* (2004) Identification of a G protein-coupled receptor specifically responsive to β -alanine. *J. Biol. Chem.* 279: 23559-23564.

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HUMAN RECOMBINANT MrgD RECEPTOR****Product No. HTS206C**

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