

**ChemiScreen[™] CALCIUM-OPTIMIZED STABLE CELL LINE
HUMAN RECOMBINANT MRGX2 MAS-RELATED RECEPTOR**

CATALOG NUMBER:	HTS058C	QUANTITY:	2 vials, 1 mL per vial
LOT NUMBER:		CONCENTRATION:	2 x 10 ⁶ cells/mL

BACKGROUND: MrgX2 is a G_q- and G_i-coupled GPCR that binds to several peptides, including the neuropeptide cortistatin-14 and the proadrenomedullin N-terminal peptide (PAMP). Dorsal root ganglia and adrenal chromaffin cells express MrgX2, and binding of these ligands to MrgX2 is thought to play a role in nociception and catecholamine release from the adrenal glands (Robas *et al.*, 2003; Kamohara *et al.*, 2005). Chemicon's cloned human MrgX2-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant MrgX2 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 MrgX2 and its ligands.

APPLICATIONS: Calcium flux assay, ligand binding assays

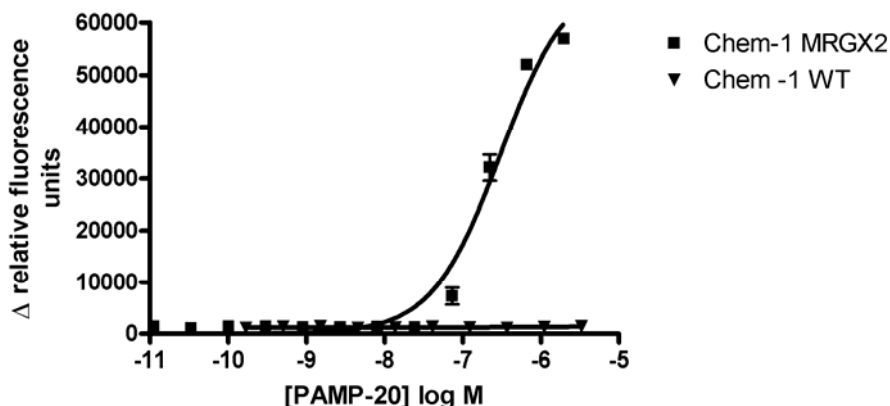


Figure 1. Calcium flux in MrgX2-expressing Chem-1 cell line induced by Proadrenomedullin Fragment 1-20 (PAMP-20). MRGX2-expressing Chem-1 (Chem-1 MRGX2) and Wild-Type Chem-1 cells (Chem-1 WT, Chemicon catalog # HTSCHEM-1) were loaded with Fluo-4 and calcium flux in response to PAMP-20 (10⁻⁵ to 10⁻¹¹ M) was determined in triplicate on a Molecular Devices Flex Station. An increase in fluorescence units of greater than 40,000 was obtained with PAMP-20.

SPECIFICATIONS: EC₅₀ for calcium mobilization by Proadrenomedullin Fragment 1-20:
~ 300 nM

HOST CELLS: Chem-1, an adherent cell line expressing the promiscuous G-protein, G_{α15}.

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TRANSFECTION: Full-length human cDNA encoding MRGX2 (Accession Number: NM_054030)

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:

Kamohara M *et al.* (2005) Identification of MrgX2 as a human G-protein-coupled receptor for proadrenomedullin N-terminal peptides. *Biochem. Biophys. Res. Commun.* 330: 1146-52.

Robas M *et al.* (2003) MrgX2 is a high potency cortistatin receptor expressed in dorsal root ganglion. *J. Biol. Chem.* 278: 44400-4.

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HUMAN RECOMBINANT MRGX2 MAS-RELATED RECEPTOR**

Product No. HTS058C

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