

**ChemiScreen™ CALCIUM-OPTIMIZED STABLE CELL LINE
HUMAN RECOMBINANT CGRP₁ CALCITONIN RECEPTOR-LIKE RECEPTOR**

CATALOG NUMBER:	HTS172C	QUANTITY:	2 vials, 1 mL per vial
LOT NUMBER:		CONCENTRATION:	2 x 10 ⁶ cells/mL

BACKGROUND: The calcitonin family of peptides, which includes calcitonin, two calcitonin-related gene peptides (CGRPs), adrenomedullin, and amylin, share a disulfide-bonded loop, an amphipathic α -helix, and an amidated C-terminus. The peptides influence a variety of physiological effects, including vascular tone, food intake and bone metabolism. The biological effects of the peptides are mediated by two class B GPCRs, CT receptor (calcitonin receptor) and CL receptor (calcitonin receptor-like receptor) (Poyner *et al.*, 2002). The pharmacology of the CT and CL receptors is strongly influenced by their association with a family of three receptor activity modifying proteins (RAMPs), which are 14-17 kD single pass transmembrane proteins. CGRP (calcitonin gene-related peptide) induces vasodilation, and its activity is primarily mediated by CGRP₁, composed of CL in complex with RAMP1 (Hasbak *et al.*, 2003). As a consequence of its role as a vasodilator, CGRP acting via CGRP₁ appears to play an important role in hypertension (Deng and Li, 2005). Chemicon's cloned human CGRP₁-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant CGRP₁ expression on the cell surface and contains high levels of the promiscuous G protein G α 15 to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for antagonists of interactions between CGRP₁ and its ligands.

APPLICATIONS: Calcium flux assay, ligand binding assays

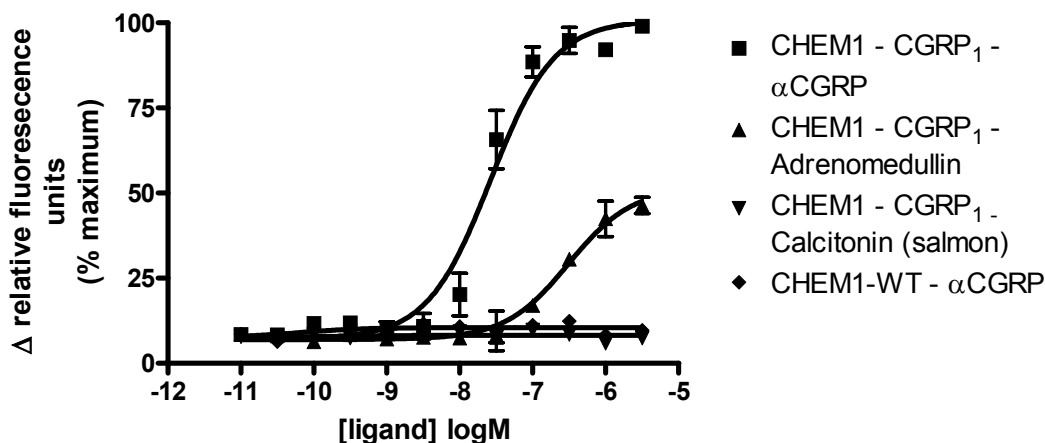


Figure 1. Calcium flux in CGRP₁-expressing Chem-1 cell line induced by α CGRP (human), Adrenomedullin, and Calcitonin (salmon). CGRP₁-expressing Chem-1 cells and Wild-Type Chem-1 cells (Chemicon catalog # HTSCHEM-1) were loaded with Fluo-4 NW and calcium flux in response to human α CGRP, Adrenomedullin, and Calcitonin (salmon) (10^{-5.5} to 10⁻¹¹M) was determined in triplicate on a Molecular Devices FLIPR^{TETRA™}.

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SPECIFICATIONS: EC50 for calcium mobilization by α CGRP: ~ 26.5 nM
EC50 for calcium mobilization by Adrenomedullin: ~ 310 nM

HOST CELLS: Chem-1, an adherent cell line expressing the promiscuous G-protein, G α 15.

TRANSFECTION: Full-length human CALCRL cDNA, encoding CGRP₁, and RAMP1 cDNA (Accession Numbers: NM_005795 and NM_005855, respectively)

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)/0.5 mg/mL Hygromycin/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:**
- Deng PY and Li YJ (2005) Calcitonin gene-related peptide and hypertension. *Peptides* 26: 1676-85.
- Hasbak P *et al.* (2003) Investigation of CGRP receptors and peptide pharmacology in human coronary arteries. Characterization with a nonpeptide antagonist. *J. Pharmacol. Exp. Ther.* 304(1):326-33.
- Poyner DR *et al.* (2002) International Union of Pharmacology. XXXII. The mammalian calcitonin gene-related peptides, adrenomedullin, amylin, and calcitonin receptors. *Pharmacol. Rev.* 54: 233-246.

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HUMAN RECOMBINANT CGRP₁ CALCITONIN RECEPTOR-LIKE RECEPTOR**

Product No. HTS172C

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