

**ChemiScreen™ CALCIUM-OPTIMIZED STABLE CELL LINE
HUMAN RECOMBINANT BB₁ BOMBESIN/NEUROMEDIN B RECEPTOR**

CATALOG NUMBER:	HTS123C	QUANTITY:	2 vials, 1 mL per vial
LOT NUMBER:		CONCENTRATION:	2 x 10 ⁶ cells/mL

BACKGROUND: Bombesin, a bioactive peptide first identified in amphibian skin, is related to two mammalian peptides, gastrin-releasing peptide (GRP) and neuromedin B (NMB). A family of 3 GPCRs, including NMB-R (BB₁), GRP-R (BB₂) and BRS-3 (BB₃), mediate the biological effects of the peptides. The receptors differ in their affinities for the peptides; BB₂ binds to GRP with 50-300-fold greater affinity than to NMB, whereas BB₁ binds to NMB with 10-800-fold greater affinity than to GRP (Tokita *et al.*, 2004). Binding of ligand to BB₁ activates G_q to increase intracellular calcium concentrations. The CNS is a major site of NMB and BB₁ expression, and BB₁ appears to be involved in thermoregulation (Ohki-Hamazaki *et al.*, 2005). Millipore's cloned human BB₁-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant BB₁ expression on the cell surface and contains high levels of the promiscuous G protein G_α15 to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for antagonists of interactions between BB₁ and its ligands.

APPLICATIONS: Calcium flux assay, ligand binding assays

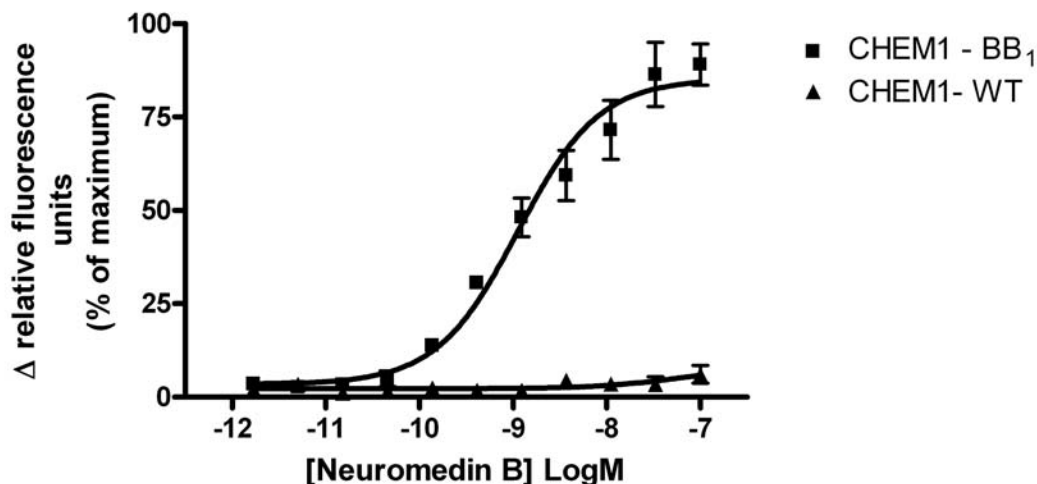


Figure 1. Calcium flux in BB₁-expressing Chem-1 cell line induced by neuromedin B. BB₁-expressing Chem-1 cells and Wild-Type Chem-1 cells (Chemicon catalog # HTSCHEM-1) were loaded with Fluo-4 and calcium flux in response to neuromedin B (10⁻⁷ to 10⁻¹² M) was determined in triplicate on a Molecular Devices FLIPR^{TETRA™}.

SPECIFICATIONS: EC50 for calcium mobilization by neuromedin B: ~ 1.1 nM
Z' = 0.83 with neuromedin B at 2x EC50

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

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TRANSFECTION: Full-length human NMBR cDNA encoding BB₁ (Accession Number: NM_002511)

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:

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:

DMEM with 4.5 g/L glucose and 4 mM glutamine
20% heat-inactivated FBS
1x NEAA
10mM HEPES
1x Pen-Strep
10% DMSO (cell culture grade)

REFERENCES:

Ohki-Hamazaki H *et al.* (2005) Development and function of bombesin-like peptides and their receptors. *Int. J. Dev. Biol.* 49: 293-300.

Tokita K *et al.* (2004) Molecular basis of the selectivity of gastrin-releasing peptide receptor for gastrin-releasing peptide. *Mol. Pharmacol.* 61: 1435-1443.

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HUMAN RECOMBINANT BB₁ BOMBESIN/NEUROMEDIN B RECEPTOR****Product No. HTS123C**

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