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ChemiScreen™ CALCIUM-OPTIMIZED STABLE CELL LINE
HUMAN RECOMBINANT PTH1 PARATHYROID HORMONE RECEPTOR

CATALOG NUMBER:	HTS030C	**QUANTITY:**	2 vials, 1 mL each
LOT NUMBER:		**CONCENTRATION:**	2 x 10^6 cells/mL

BACKGROUND: Parathyroid hormone (PTH) plays a critical role in mineral ion homeostasis, particularly in bone and kidney (Mannstadt *et al.*, 1999; Gensure *et al.*, 2005). A related peptide, parathyroid hormone-related peptide (PTHrP), plays an important role in skeletal development. Both peptides exert their biological actions by binding to a class B GPCR, PTH1, that signals primarily by activation of adenylate cyclase (Schipani *et al.*, 1993). Mutations in PTH1 have been identified as the cause of Jansen's metaphyseal chondrodysplasia, Blomstrand's chondrodysplasia, and enchondromatosis (Schipani and Provot, 2003). Intermittent treatment with PTH has important clinical utility in building bone mass in patients with osteoporosis (Rosen, 2003). Millipore's cloned human PTH1-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant PTH1 expression on the cell surface and contains high levels of the promiscuous G protein Gα15 to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for antagonists of interactions between PTH1 and its ligands.

APPLICATIONS: Calcium flux assay, ligand binding assays

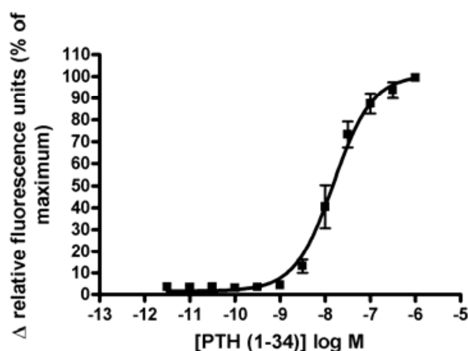

Figure 1. Calcium flux in PTH1–expressing Chem-1 cell line induced by PTH. PTH1–expressing Chem-1 cells were loaded with Fluo-4 and calcium flux in response to recombinant human PTH (10^{-6} to $10^{-11.5}$ M) was determined in triplicate on a Molecular Devices Flex Station.

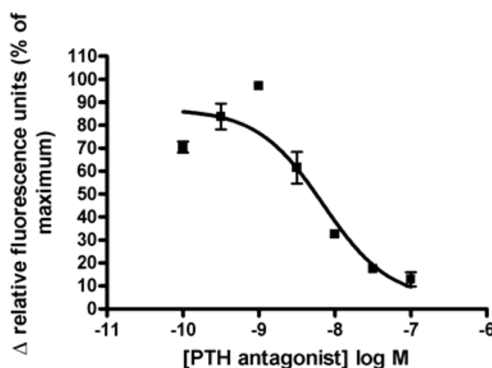

Figure 2. Inhibition of PTH-mediated calcium flux in PTH1-expressing Chem-1 cells by a PTH antagonist. PTH1–expressing Chem-1 cells were loaded with Fluo-4, washed, and preincubated with the indicated concentration of [D-Trp^{12}, Tyr^{34}]-bPTH(7-34) for 10 min. Calcium flux in response to 13 nM PTH was determined on a Molecular Devices Flex Station. An IC50 of 7 nM for [D-Trp^{12}, Tyr^{34}]-bPTH(7-34) was obtained.

SPECIFICATIONS: EC50 for calcium mobilization by PTH: ~ 13 nM
IC50 for [D-Trp^{12}, Tyr^{34}]-bPTH(7-34): 7-15 nM
Signal:noise at PTH E_{max}: 723

HOST CELLS: Chem-1, an adherent cell line with endogenous Gα15 expression

TRANSFECTION: Full-length human PTHR1 cDNA encoding PTH1 (Accession Number: NM_000316)

PRESENTATION:

Cells are frozen at 2 x 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 37ºC 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 37ºC with 5% CO_2.
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 37ºC with 5% CO_2 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 x 10^6 cells/mL in Chem-1 Freezing Media (cell densities of 2-10 x 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:

Gensure RC *et al.* (2005) Parathyroid hormone and parathyroid hormone-related peptide, and their receptors. *Biochem. Biophys. Res. Commun.* 328: 666-78

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Rosen CJ (2003) The cellular and clinical parameters of anabolic therapy for osteoporosis. *Crit. Rev. Eukaryot. Gene Expr.* 13: 25-38.

Schipani E and Provot S. (2003) PTHrP, PTH, and the PTH/PTHrP receptor in endochondral bone development. *Birth Defects Res. C. Embryo Today* 69: 352-62

Schipani E (1993) Identical complementary deoxyribonucleic acids encode a human renal and bone parathyroid hormone (PTH)/PTH-related peptide receptor. *Endocrinology* 132: 2157-65

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HUMAN RECOMBINANT PTH1 PARATHYROID HORMONE RECEPTOR

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