

**READY-TO-ASSAY™ CALCIUM-OPTIMIZED CELLS
HUMAN RECOMBINANT PTH₂ PARATHYROID HORMONE RECEPTOR**

CATALOG NUMBER: HTS173F **QUANTITY:** 1 vial, 1 mL
LOT NUMBER: **CONCENTRATION:** 1 x 10⁷ viable cells/mL

BACKGROUND: Millipore's Ready-To-Assay™ Calcium-Optimized Cells are GPCR-expressing cell lines that are designed for simple, rapid calcium assays with no requirement for culturing cells. The user simply thaws the cells with maximal viability, dispenses into assay plates, and assays for calcium response the next day.

The Ready-To-Assay™ cells are derived from ChemiScreen™ calcium-optimized stable cell lines, which express the GPCR target of interest at high levels on the cell surface, in a host cell line containing high levels of the promiscuous Gα15 protein to couple the receptor to the calcium signaling pathway. The Ready-To-Assay™ cells are prepared by chemical treatment at a concentration optimized for effective growth arrest while maintaining high viability (>80%) after thawing and overnight plating. Pharmacological functionality of the Ready-To-Assay™ cells is identical to that of the originating GPCR cell line.

Parathyroid hormone (PTH) is recognized by three different class B GPCRs; PTH₁, PTH₂, and PTH₃, which couple to G_s to stimulate cAMP production. The PTH₁ receptor is found in high levels in the kidney and bone, where it binds PTH and PTHrP to regulate Ca²⁺ homeostasis (Brown *et al.*, 1996). The PTH₂ receptor, which binds PTH and the neuropeptide tuberoinfundibular peptide 39 (TIP-39) but not PTHrP, has about 50% amino acid sequence identity to the PTH₁ receptor. PTH₂ is found in the greatest volume in the nervous system and at a very low density in the kidney and bone (Usdin *et al.*, 1995). The PTH₃ receptor, which has been recently discovered in the zebrafish, has 61% amino acid identity to the zebrafish PTH₁ receptor and also seems to share its ligand affinity (Rubin *et al.*, 1999). Studies with the selective PTH₂ receptor agonist, TIP-39, and the distribution of the receptor in the superficial dorsal horn of the spinal cord suggest the receptor may play a role in pain perception (Usdin *et al.*, 1999). Millipore's cloned human PTH₂-expressing cell line is made in the Chem-1 host, an adherent cell line that supports high levels of recombinant rhesus PTH₂ expression on the cell surface and contains high levels of promiscuous G protein to couple the receptor to the calcium signaling pathway. The untreated human PTH₂-Chem-1 cell line and the Ready-To-Assay™ human PTH₂ cells have equivalent EC₅₀s for TIP-39.

APPLICATIONS: Calcium flux assay

SPECIFICATIONS:

	EC ₅₀ for TIP-39 (nM)	Maximum Signal (RFU)	Z'
Ready-To-Assay Cells	10.95	3299	0.79
Continuous Passage Cells	12.41	2119	0.63

HOST CELLS: Chem-1 an adherent cell line expressing a recombinant promiscuous G-protein.

TRANSFECTION: Full-length human PTHR2 cDNA encoding PTH₂ (Accession Number: NM_005048)

PLATING 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)
100 U/mL Pen-Strep (from 100x stock, Millipore TMS-AB2-C)

PRESENTATION:

Cells are frozen at 1×10^7 cells/mL in DMEM/20% fetal bovine serum/100 U/ml penicillin and streptomycin/10% DMSO.

STORAGE:

Place cells in liquid nitrogen immediately upon receipt. Maintain frozen in liquid nitrogen for up to 5 years.

ASSAY PROTOCOL:

- 1) 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.
- 2) Transfer contents of the vial to a sterile 15 mL conical tube. Add 10 mL prewarmed plating media to the cells and mix gently to resuspend cells. Centrifuge at 200 x g. Remove all but 0.5 mL media.
- 3) Resuspend cells to 0.5×10^6 cells/mL in plating media. Dispense the cell suspension into a 96-well assay plate at 200 μ L per well to obtain a density of approximately 1×10^5 cells/well.
- 4) Place the assay plate in a humidified 37°C incubator with 5% CO₂.
- 5) The cells may be assayed 16-24 hours after plating. It is recommended to wash the cells with assay buffer at least once prior to addition of loading dye.

REFERENCES:

Brown EM *et al.*, (1996) Serpentine receptors for parathyroid hormone, calcitonin and extracellular calcium ions. *Bailliere's Clin. Endocrinol. Metab.* 10:123–161.

Usdin, TB *et al.*, (1995) The PTH₂ receptor and TIP39: a new peptide–receptor system *J. Biol. Chem.* 270: 15455-15458

Rubin DA *et al.*, (1999) Zebrafish express the common parathyroid hormone/parathyroid hormone-related peptide receptor (PTH1R) and a novel receptor (PTH3R) that is preferentially activated by mammalian and fugu fish parathyroid hormone-related peptide. *J. Biol. Chem.* 274:28185–28190.

Usdin, TB *et al.*, (1999) TIP39: a new neuropeptide and PTH₂-receptor agonist from hypothalamus. *Nat Neurosci.* 2: 941-943

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HUMAN RECOMBINANT PTH₂ PARATHYROID HORMONE RECEPTOR**

Product No. HTS173F

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