

**ChemiScreen™ CALCIUM-OPTIMIZED STABLE CELL LINE
HUMAN RECOMBINANT PRP/GPR10 PROLACTIN-RELEASING PEPTIDE RECEPTOR**

CATALOG NUMBER:	HTS057C	QUANTITY:	2 vials, 1 mL per vial
LOT NUMBER:		CONCENTRATION:	2 x 10 ⁶ cells/mL

BACKGROUND: PRP, encoded by the GPR10 (or hGR3) gene, is a G_q-coupled receptor for prolactin-releasing peptide that is expressed in the pituitary (Hinuma *et al.*, 1998). Genetic studies in rodents indicate that lack of GPR10 leads to hyperphagia, obesity and dyslipidemia (Gu *et al.*, 2004; Watanabe *et al.*, 2005). In humans, genetic variations in GPR10 are associated with lowered blood pressure (Bhattacharyya *et al.*, 2003). Chemicon's cloned human PRP-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant PRP 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 PRP and its ligands.

APPLICATIONS: Calcium flux assay, ligand binding assays

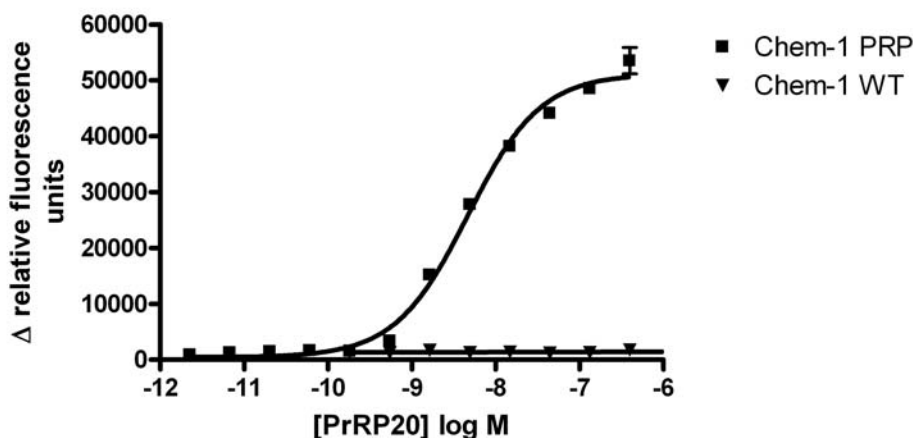


Figure 1. Calcium flux in PRP-expressing Chem-1 cell line induced by Prolactin-releasing Peptide Fragment 12-31. PRP-expressing Chem-1 (Chem-1 PRP) and Wild-Type Chem-1 cells (Chem-1 WT, Chemicon catalog # HTSCHEM-1) were loaded with Fluo-4 and calcium flux in response to PRP Fragment 12-31 (10⁻⁶ to 10⁻¹² M) was determined in triplicate on a Molecular Devices Flex Station. An increase in fluorescence units of greater than 40,000 was obtained with PRP Fragment 12-31.

SPECIFICATIONS: EC50 for calcium mobilization by PRP Fragment 12-31: ~ 5 nM

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

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TRANSFECTION: Full-length human GPR10 cDNA encoding PRP (Accession Number: NM_004248)

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:

Bhattacharyya S *et al.* (2003) Association of polymorphisms in GPR10, the gene encoding the prolactin-releasing peptide receptor with blood pressure, but not obesity, in a U.K. Caucasian population. *Diabetes* 52: 1296-9.

Gu W *et al.* (2004) The prolactin-releasing peptide receptor (GPR10) regulates body weight homeostasis in mice. *J. Mol. Neurosci.* 22: 93-103.

Hinuma S *et al.* (1998) A prolactin-releasing peptide in the brain. *Nature* 393: 272-6.

Watanabe TK *et al.* (2005) Mutated G-protein-coupled receptor GPR10 is responsible for the hyperphagia/dyslipidaemia/obesity locus of Dmo1 in the OLETF rat. *Clin. Exp. Pharmacol. Physiol.* 32: 355-66.

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HUMAN RECOMBINANT PRP/GPR10 PROLACTIN-RELEASING PEPTIDE RECEPTOR**

Product No. HTS057C

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