Human Recombinant Prolactin-releasing Peptide Receptor Stable Cell Line

Technical Manual No. TM0583 **Version 10132010**

I. Introduction

Catalog Number: M00341

Cell Line Name: CHO-K1/PRLHR

Gene Synonyms: PRLHR; GPR10; GR3; MGC126539; MGC126541; PrRPR

Expressed Gene: Genbank Accession Number NM_004248; no expressed tags

Host Cell: CHO-K1

Quantity: Two vials of frozen cells (3×10^6 per vial)

Stability: 16 passages

Application: Functional assay for PRLHR receptor

Freeze Medium: 45% culture medium, 45% FBS, 10% DMSO

Complete Growth Medium: Ham's F12, 10% FBS

Culture Medium: Ham's F12, 10% FBS, 400 µg/ml G418

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. Background

The prolactin releasing hormone receptor PRLHR also named PrRP receptor is a G-protein coupled receptor that binds the prolactin releasing hormone. RT-PCR analysis showed expression of PRLHR in the human brain, pituitaries, normal portions of adrenal glands and various tumor tissues. Northern blot analysis showed high expression of PRLHR only in tumor tissues of pheochromocytomas, indicating that PRLHR expression is high in pheochromocytomas. The present study has shown that PRLHR mRNA was widely expressed in the brain tissues, pituitaries, adrenal glands and various tumors. The high expression of PRLHR receptor in pheochromocytomas suggests potential pathophysiological roles of PRLHR in these tumors

§: GenScript employs a PCR-based method to test the mycoplasma. The test covers 11 of the most common strains of mycoplasma, (covering approximately 95% of M. fermentans, M. hyorhinis, M. arginini, M. orale, M. salivarium, M. hominis, M. pulmonis, M. arthritidis, M. neurolyticum, M. hyopneumoniae and M. capricolum) and one species Ureaplasma (U. urealyticum), with sufficient sensitivity and specificity.

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III. Representative Data

Concentration-dependent stimulation of intracellular calcium mobilization by PrRP31 in CHO-K1/PRLHR and CHO-K1 cells

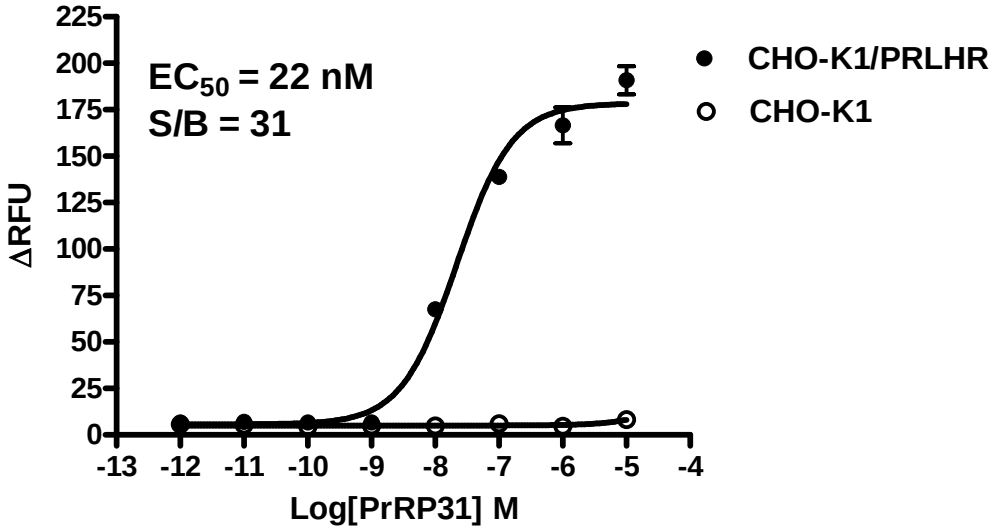

Figure 1. PrRP31-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/PRLHR and CHO-K1 cells. The cells were loaded with Calcium-4 prior to stimulation with a PRLHR receptor agonist, PrRP31. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of PrRP31 (Mean ± SD, n = 2). The EC_{50} of PrRP31 on PRLHR in CHO-K1 cells was 22 nM. The S/B of PrRP31 on PRLHR in CHO-K1 cells was 31.

Notes:

1. EC_{50} value is calculated with four parameter logistic equation:

   Y=Bottom + (Top-Bottom)/(1+10^((LogEC_{50}-X)*HillSlope))

   X is the logarithm of concentration. Y is the response

   Y is RFU and starts at Bottom and goes to Top with a sigmoid shape.

2. Signal to background Ratio (S/B) = Top/Bottom

IV. Thawing and Subculturing

Thawing protocol

1. Remove the vial from liquid nitrogen tank and thaw cells quickly in a 37°C water-bath.
2. Just before the cells are completely thawed, decontaminate the outside of the vial with 70% ethanol and transfer the cells to a 15 ml centrifuge tube containing 9 ml of complete growth medium.
3. Pellet cells by centrifugation at 200 x g force for 5 min, and discard the medium.
4. Resuspend the cells in complete growth medium.
5. Add 10 ml of the cell suspension in a 10 cm dish.
6. Add G418 to a concentration of 400 μg/ml the following day.

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Subculturing protocol

1. Remove and discard culture medium.
2. Wash cells with PBS (pH=7.4) to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 ml of 0.05% (w/v) Trypsin- EDTA (GIBCO, Cat No. 25300) solution to 10 cm dish and observe the cells under an inverted microscope until cell layer is dispersed (usually within 3 to 5 minutes).
   Note: To avoid clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting, centrifuge the cells 200 x g force for 5min, and discard the medium.
5. Resuspend the cells in culture medium and add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: 1:3 to 1:8 weekly.
Medium Renewal: Every 2 to 3 days

V. References

1. Marchese A *et al.* (1996) Cloning and chromosomal mapping of three novel genes, GPR9, GPR10, and GPR14, encoding receptors related to interleukin 8, neuropeptide Y, and somatostatin receptors. *Genomics* 29 (2): 335–44.
2. Takahashi K *et al.* (2003) Expression of prolactin-releasing peptide and its receptor in the human adrenal glands and tumor tissues of adrenocortical tumors, pheochromocytomas and neuroblastomas. *Peptides* 23 (6): 1135–40

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