Human Recombinant OX2 Orexin Receptor Stable Cell Line

Cat. No. M00316 **Version 06092014**

I. INTRODUCTION

Catalog Number: M00316

Cell Line Name: CHO-K1/OX2

Gene Synonyms: OX2R; HCRTR2

Expressed Gene: Genbank Accession Number NM_001526; 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 OX2 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

Hypocretin (orexin) receptor 2, also known as OX2, is a human protein encoded by the HCRTR2 gene. Orexin A and orexin B are neuropeptides originally identified as endogenous ligands for OX2. Orexin neuropeptides are produced by a small group of neurons in the lateral hypothalamic and perifornical areas, a region that is classically implicated with the control of mammalian feeding behavior. Orexin neurons that project throughout the central nervous system to nuclei are known to be important in the control of feeding, sleep-wakefulness, neuroendocrine homeostasis, and autonomic regulation.

§: 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.

REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by Orexin A in CHO-K1/OX2 and CHO-K1 cells

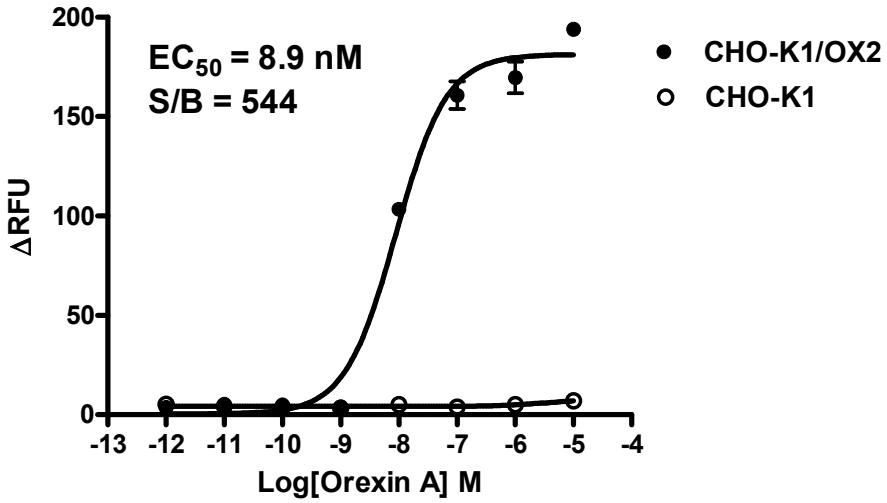

Figure 1. Orexin A-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/OX2 and CHO-K1 cells. The cells were loaded with Calcium-4 prior to stimulation with an OX2 receptor agonist, Orexin A. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of Orexin A (Mean ± SD, n = 2). The EC_{50} of Orexin A on OX2 in CHO-K1 cells was 8.9 nM. The S/B of Orexin A on OX2 in CHO-K1 cells was 544.

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

III. 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 remove the medium.
4. Resuspend the cells in complete growth medium.
5. Transfer the cell suspension to a 10 cm dish with 10 ml of complete growth medium.
6. Grow the cells in incubator with 37°C, 5 %CO_2.
7. Add antibiotic in the following day.

Sub-culturing Protocol

1. Remove the culture medium from cells.
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 into 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 cells clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells detach. If cells are difficult to detach, please place the dish in 37°C incubator for ~2 min.
4. Add 6.0 to 8.0 ml of complete growth medium into dish and aspirate cells by gently pipetting.
5. Centrifuge the cells at 200 x g force for 5min, and remove the medium.
6. Resuspend the cells in culture medium and add the cells suspension to new culture dish.
7. Grow the cells in incubator with 37°C, 5 %CO_2.

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

IV. REFERENCES

1. Sakurai, T., Amemiya, A., Ishii, M., *et al*. (1998) Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell*, 92. 573-585.
2. Ammoun, S., Holmqvist, T., Shariatmadari, R., *et al*. (2003) Distinct recognition of OX1 and OX2 receptors by orexin peptides. *J Pharmacol Exp Ther*, 305. 507-514.
3. Smart, D., Jerman, J. C., Brough, S. J., *et al*. (1999) Characterization of recombinant human orexin receptor pharmacology in a Chinese hamster ovary cell-line using FLIPR. *Br J Pharmacol*, 128. 1-3.

GenScript USA Inc,
860 Centennial Ave.
Piscataway, NJ 08854
Toll-Free: 1-877-436-7274
Tel: 1-732-885-9188, Fax: 1-732-210-0262
Email: product@genscript.com
Web: http://www.genscript.com

For Research Use Only.

Limited Use License Agreement

This is a legal agreement between you (Licensee) and GenScript USA Inc. governing use of GenScript's stable cell line products and protocols provided to licensee. By purchasing and using the stable cell line, the buyer agrees to comply with the following terms and conditions of this label license and recognizes and agrees to such restrictions:

1) The products are not transferable and will be used at the site where they were purchased. Transfer to another site owned by buyer will be permitted only upon written request by buyer followed by subsequent written approval by GenScript.
2) The purchaser cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party.
3) The products sold by GenScript are for laboratory and animal research purposes only. The products are not to be used on humans, for consumption, or for any unlawful uses.

GenScript USA Inc. will not assert against the buyer a claim of infringement of patents owned or controlled by GenScript USA Inc. and claiming this product based upon the manufacture, use or sale of a clinical diagnostic, therapeutic and vaccine, or prophylactic product developed in research by the buyer in which this product or its components has been employed, provided that neither this product nor any of its components was used in the manufacture of such product. For information on the use of this product for other purposes, contact Marketing Department, GenScript USA Inc., 120 Centennial Avenue, Piscataway, New Jersey 08840, U.S.A. Phone: 1-732-885-9188. Fax: 1-732-210-0262. Email: marketing@genscript.com.