Human Recombinant OX1 Orexin Receptor Stable Cell Line

Cat. No. M00224 **Version 06092014**

I. INTRODUCTION

Catalog Number: M00224
Cell Line Name: CHO-K1/OX1
Gene Synonyms: OX1, HRTR1
Expressed Gene: Genbank Accession Number NM_001525; 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 OX1 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 orexin/hypocretin peptides orexin A and orexin B (also known as hcrt-1 and hcrt-2) are 33- and 28-amino acid peptides, respectively. They are preferentially expressed in hypothalamus. The orexins have a range of physiological functions including feeding control, energy metabolism, neuroendocrine function modulation, and sleep-wake cycle regulation. The two orexin receptor subtypes OX1 and OX2 both mediate the action of orexin-A and orexin-B and couple efficiently through Gq/11 to activate phospholipase C and lead to elevation of intracellular calcium.

§: 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.

III. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by Orexin A in CHO-K1/OX1 and CHO-K1 cells

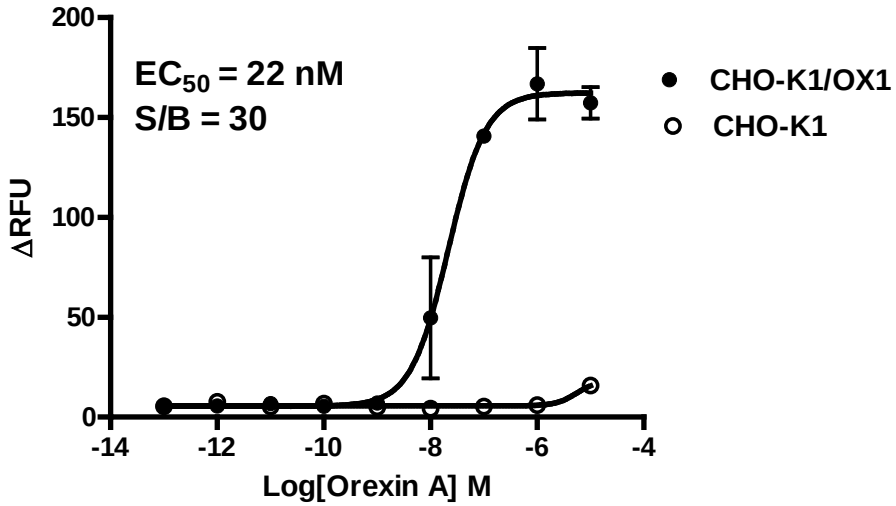

Figure 1. Orexin A-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/OX1 and CHO-K1 cells. The cells were loaded with Calcium-4 prior to stimulation with a OX1 receptor agonist, Orexin A. The intracellular calcium change was measured by FlexStation. The relative 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 OX1 in CHO-K1 cells was 22 nM. The S/B of Orexin A on OX1 in CHO-K1 cells was 30.

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 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

V. REFERENCES

1. Esmaeil Akbari, Nasser Naghdi and Fereshteh Motamedi (2006) Functional inactivation of orexin 1 receptors in CA1 region impairs acquisition, consolidation and retrieval in Morris water maze task. *Behavioural Brain Research* 173(1): 47-52
2. Kukkonen JP et al. (2002) Functions of the orexinergic/hypocretinergic system *Am. J. Physiol*. 283: C1567-C1591
3. Sakurai, T., Amemiya, A., Ishii, M., Matsuzaki, I., Chemelli, R.M., Tanaka, H., Williams, S.C., Richardson, J.A., Kozloski, G.P., Wilson, S., Arch, J.R.S., Buckingham, R.C., Haynes, A.C., Carr, S.A., Annan, R.S., Mcnulty, D.E., Liu,W.S., Terrett, J.A., Elshourbagy, N.A., Bergsma, D.J. and Yangisawa, M. (1998) Orexins and orexin receptors: a family of hypothalamic neuropeptides and G-protein coupled receptors that regulate feeding behaviour. *Cell* 92: 573−585

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