
Human Recombinant OX2 Orexin Receptor Stable Cell Line

Cat. No. M00316

Version 07292020

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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

Culture Properties: Adherent

Quantity: Two vials of frozen cells (>1×10⁶ per vial)

Stability: More than 16 passages

Application: Functional assay for OX2 receptor (Calcium flux assay)

Freeze Medium: 45% culture medium, 45% FBS (Cat. #10099-141, Gibco), 10% DMSO (Cat. #D2650, Sigma)

Complete Growth Medium: Ham's F-12K (Kaighn's) (Cat. #21127, Life Technologies), 10% FBS

Culture Medium: Ham's F-12K (Kaighn's), 10% FBS, 400 µg/ml G418 (Cat. #10131-035, Gibco)

Mycoplasma Status: Negative*

Storage: Liquid nitrogen immediately upon receipt.

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.

* The mycoplasma test was performed with MycoAlert™ PLUS Mycoplasma Detection Kit of Lonza.

III. REPRESENTATIVE DATA

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

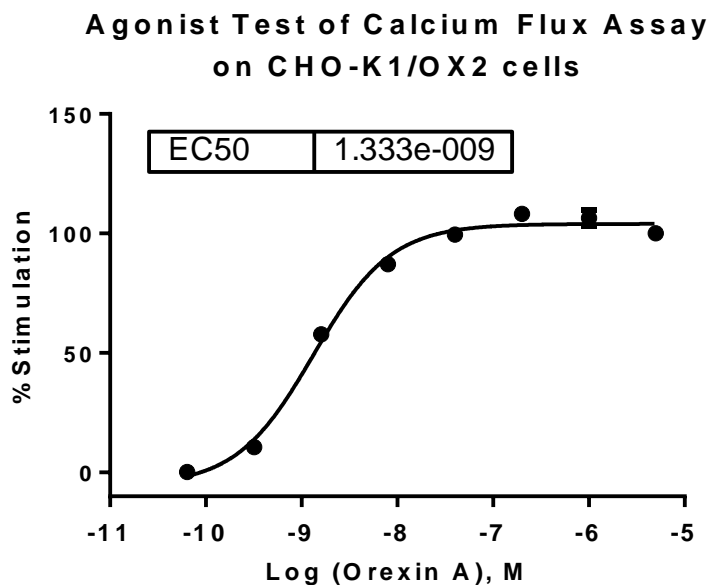


Figure 1. Orexin A-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/OX2 cells. The cells were loaded with Calcium-4 prior to being stimulated with agonist orexin A. The intracellular calcium change was measured by FLIPR. The relative fluorescent units (RFU) were normalized and plotted against the log of the cumulative doses of orexin A (Mean \pm SD, n = 2). The EC₅₀ of orexin A on this cell was 1.3 nM.

Notes:

- EC₅₀ value is calculated with four parameter logistic equation:

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{((\text{LogEC}_{50} - X) * \text{HillSlope}))}$$
 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.

IV. THAWING AND SUBCULTURING

Thawing Protocol

- Remove the vial from liquid nitrogen tank and thaw cells quickly in a 37°C water-bath.
- 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.
- Pellet cells by centrifugation at 200 x g force for 5 min, and remove the medium.
- Resuspend the cells in complete growth medium.
- Transfer the cell suspension to a 10 cm dish with 10 ml of complete growth medium.
- Grow the cells in incubator with 37°C, 5 %CO₂.

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 %CO₂.

Subcultivation Ratio: 1:3 to 1:8

Medium Renewal: Every 2 to 3 days

V. 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.

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