
Human Recombinant OX1 Orexin Receptor Stable Cell Line**Cat. No. M00224****Version 07272020**

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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

Culture Properties: Adherent

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

Stability: More than 16 passages

Application: Functional assay for OX1 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 F12K (Kaighn's) (Cat. #21127-022, Gibco), 10% FBS

Culture Medium: Ham's F12K (Kaighn's), 10% FBS, 400 µg/ml G418 (Cat. #10131-035, Gibco)

Mycoplasma Status: Negative*

Storage: Liquid nitrogen immediately upon receipt

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.

* 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/OX1 cells

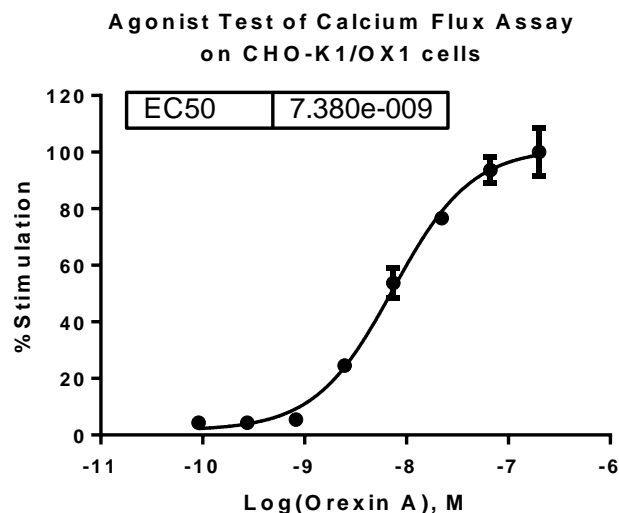


Figure 1: Orexin A-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/OX1 cells. The cells were loaded with Calcium-4 prior to being stimulated with OX1 receptor agonist, orexin A. The intracellular calcium change was measured by FLIPR. The relative fluorescent units (RFU) were normalized and plotted against the log of the cumulative doses (5-fold dilution) of orexin A (Mean \pm SD, n = 2). The EC₅₀ of orexin A on CHO-K1/OX1 cells was 7.4 nM.

Note:

1. 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 and starts at Bottom and goes to Top with a sigmoid shape.

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 for 5 min, and remove the medium.
4. Resuspend the cells with 1 ml complete growth medium.
5. Transfer the cell suspension to a 10 cm dish containing 10 ml complete growth medium.
6. Transfer the dish into an incubator of 37°C, 5% CO₂.
7. Add antibiotic into the medium on the next 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 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 during incubation. 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 complete growth medium into dish and aspirate cells by gently pipetting.
5. Centrifuge the cells at 200 X g for 5min, and remove the medium.
6. Resuspend the cells in culture medium and transfer the cells to a new culture dish.
7. Transfer the dish into an incubator of 37°C, 5% CO₂.

Subcultivation Ratio: 1:3 to 1:8

Medium Renewal: Every 2 to 3 days

V. REFERENCES

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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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