I. Introduction

Catalog Number: M00195

Cell Line Name: CHO-K1/OXTR

Gene Synonyms: OT, OT-R

Expressed Gene: Genbank Accession Number NM_000916; 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 OXTR 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

Oxytocin is an abundant neuropeptide that exerts a wide spectrum of central and peripheral effects. In the context of human reproduction, oxytocin promotes uterine contractions and lactation. It is the most commonly used drug for labor induction. Oxytocin receptors are expressed in the uterus and in mammary glands, where they mediate functions related to parturition, such as contraction of the uterine myometrium during labor and milk letdown. In addition, oxytocin receptors are expressed in a variety of other peripheral tissues and in the brain, where oxytocin mediates a variety of functions. OXTR antagonists may be promising candidates to prevent preterm labor and dysmenorrheal and its agonists may also be useful for treatment of psychiatric illnesses such as anxiety, drug abuse, sexual dysfunctions, eating disorders, and autism.
### III. Representative Data

Concentration-dependent stimulation of intracellular calcium mobilization by Oxytocin in CHO-K1/OXTR and CHO-K1 cells

**Figure 1.** Oxytocin-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/OXTR and CHO-K1 cells. The cells were loaded with Calcium-4 prior to stimulation with an OXTR receptor agonist, Oxytocin. 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 Oxytocin (Mean ± SD, n = 2). The EC$_{50}$ of Oxytocin on OXTR in CHO-K1 cells was 0.0087 nM. The S/B of Oxytocin on OXTR in CHO-K1 cells was 11.

**Notes:**
1. EC$_{50}$ value is calculated with four parameter logistic equation:
   \[ Y = \text{Bottom} + \frac{(\text{Top} - \text{Bottom})}{(1 + 10^{((\text{LogEC}_{50} - X) \times \text{HillSlope})})} \]
   
   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.
2. Signal to background Ratio (S/B) = Top/Bottom

### IV. Thawing and Subculturing

**Thawing:**
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.
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 5 min, 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

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., 860 Centennial Avenue, Piscataway, New Jersey 08840, U.S.A. Phone: 1-732-885-9188. Fax: 1-732-210-0262. Email: marketing@genscript.com.