

Human Recombinant Endothelin Receptor Type B Stable Cell Line**Cat. No. M00150****Version 05262014**

I	Introduction	1
II	Background.....	1
III	Representative Data.....	2
IV	Thawing and Subculturing.....	2
V	References	3
	Limited Use License Agreement	4

I. INTRODUCTION

Catalog Number: M00150

Cell Line Name: CHO-K1/EDNRB

Expressed Gene: GenBank accession number NM_000115, no expressed tags

Host Cell: CHO-K1

Quantity: Two vials of frozen cells (3×10^6 per vial)

Stability: 16 passages

Application: Functional assays for EDNRB 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, 200 μ g/ml Zeocin

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

In mammals, the endothelin (ET) family comprises three endogenous isoforms: ET-1, ET-2, and ET-3. These endothelium-derived peptides perform their functions via two endothelin receptors, classified as EDNRA and EDNRB. EDNRB receptors present on the endothelial cells lining the vessel wall and play a role in the release of endothelium-derived relaxing factors such as nitric oxide (NO) and prostanoids and in removing ET from the circulation.

§: 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 ET-1 in CHO-K1/EDNRB and CHO-K1 cells

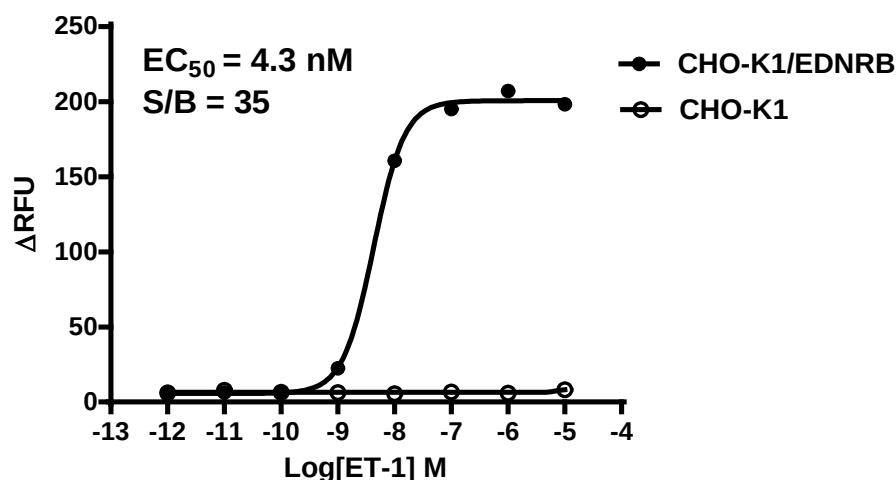


Figure 1. ET-1-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/EDNRB and CHO-K1 cells. The cells were loaded with Calcium-4 prior to stimulation with an EDNRB receptor agonist, ET-1. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of ET-1 (Mean $\pm$ SD, n = 2). The EC₅₀ of ET-1 on EDNRB in CHO-K1 cells was 4.3 nM. The S/B of ET-1 on EDNRB in CHO-K1 cells was 35.

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.
- Signal to background Ratio (S/B) = Top/Bottom

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.
- Add antibiotic in the following day.

Sub-culturing Protocol

860 Centennial Ave., Piscataway, NJ 08854, USA

Toll-Free: 1-877-436-7274 Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: product@genscript.com Web: www.genscript.com

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 weekly.

Medium Renewal: Every 2 to 3 days

V. REFERENCES

1. Warner, T. D., de Nucci, G. and Vane, J. R. (1989) Rat endothelin is a vasodilator in the isolated perfused mesentery of the rat. *Eur. J. Pharmacol.*, 159, 325 - 326.
2. Fukuroda, T. (1994) Clearance of circulating endothelin-1 by ETB receptors in rats. *Biochem. Biophys. Res. Commun.*, 199, 1461 – 1465
3. Haendler, B. (1992) Molecular cloning of human endothelin (ET) receptors ETA and ETB. *J. Cardiovasc. Pharmacol.* 20 SUPPL 12, S1-S4 (1992)

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.