Human Recombinant Endothelin Receptor Type B Stable Cell Line
Cat. No. M00150 **Version 06122020**

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
Culture Properties: Adherent
Quantity: Two vials of frozen cells (>1×10^6 per vial)
Stability: More than 16 passages
Application: Functional assays for EDNRB 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, 200 μg/ml Zeocin (Cat. #R250-01, Life Technologies)
Mycoplasma Status: Negative*
Storage: Liquid nitrogen immediately upon receipt

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.

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

III. REPRESENTATIVE DATA

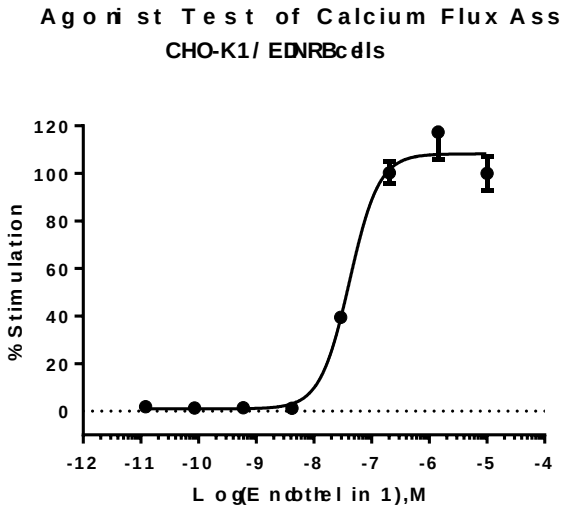

Figure 1: ET-1-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/EDNRB cells. The cells were loaded with Calcium-4 prior to stimulation with an EDNRB receptor agonist ET-1. The intracellular calcium change was measured by FLIPR^{TETRA}. The relative fluorescent units (RFU) were normalized and plotted against the log of the cumulative doses of ET-1 (Mean ± SD, n = 2). The EC_{50} of ET-1 on EDNRB expressing CHO-K1 cells was 41.5 nM.

Note:

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 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 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% CO_2.
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% CO_2.

Subcultivation Ratio: 1:3 to 1:8
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)

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