Human Recombinant B2 Bradykinin Receptor Stable Cell Line

Technical Manual No. TM0407 **Version 10132010**

I. Introduction

Catalog Number: M00184

Cell Line Name: CHO-K1/B2/Gα15

Gene Synonyms: BDKRB2, B2R, BK2, BK-2, BKR2, BRB2, DKFZp686O088

Expressed Gene: Genbank Accession Number NM_000623; no expressed tags

Host Cell: CHO-K1/Gα15

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

Stability: 16 passages

Application: Functional assay for B2 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, 100 μg/ml Hygromycin B

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. Background

Bradykinin (BK) is a pro-inflammatory polypeptide that can cause pain, inflammation, increased vascular permeability, vasodilation, contraction of various smooth muscles, and cell proliferation by stimulating B1 and B2 receptors. B2 receptors are most commonly distributed in the vascular and non-vascular smooth muscle and in the heart. The B2 receptor mediates the action of bradykinin (BK) and lysyl-bradykinin (Lys-BK). The stimulation of BK B2 receptors is not only implicated in the pathogenesis of inflammation, pain, and tissue injury but also in cardioprotective mechanisms. So B2 receptor agonists may have important clinical value in the treatment and prevention of various cardiovascular disorders such as hypertension, ischaemic heart disease, left ventricular hypertrophy, ventricular remodeling, congestive heart failure, and diabetic disorders.

§: 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.

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III. Representative Data

Concentration-dependent stimulation of intracellular calcium mobilization by Bradykinin in CHO-K1/B2/Gα15 and CHO-K1/Gα15 cells

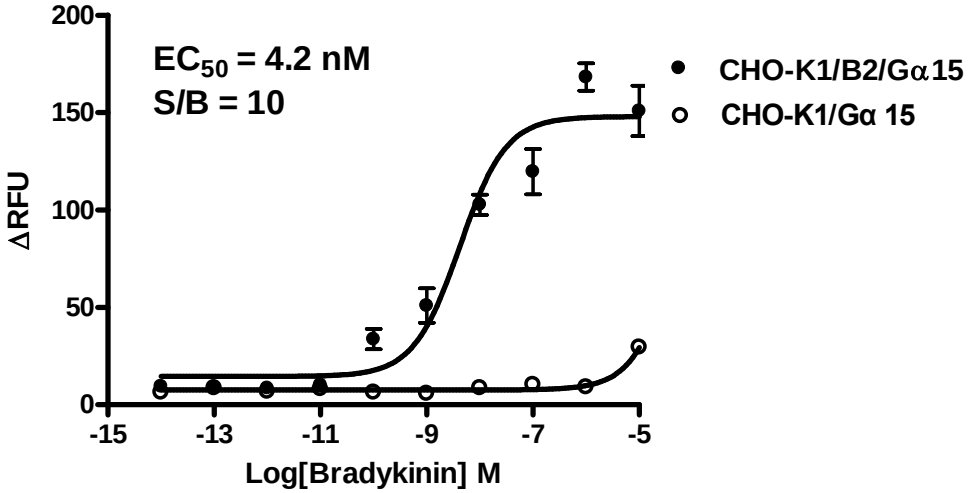

Figure 1. Bradykinin-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/B2/Gα15 and CHO-K1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with a B2 receptor agonist, Bradykinin. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of Bradykinin (Mean ± SD, n = 2). The EC_{50} of Bradykinin on B2 co-expressing with Gα15 in CHO-K1 cells was 4.2 nM. The S/B of Bradykinin on B2 co-expressing with Gα15 in CHO-K1 cells was 10.

Notes:

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

   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: 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 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 Hygromycin B and G418 to concentrations of 100 µg/ml and 400 µg/ml respectively the following day.

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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 5min, 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

1. Sharma, J.N. (2003) Bradykinin receptor antagonists: therapeutic implications. *IDrugs.* 6(6):581-6

2. Heitsch, H. (2003) The therapeutic potential of bradykinin B2 receptor agonists in the treatment of cardiovascular disease. *Expert Opin Investig Drugs.*12(5):759-70

3. Leeb-Lundberg, L.M., (2005) International union of pharmacology. XLV. Classification of the kinin receptor family: from molecular mechanisms to pathophysiological consequences. *Pharmacol Rev.* 57(1):27-77

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