Human Recombinant TP Prostanoid Receptor Stable Cell Line

Technical Manual No. TM0505 **Version 10132010**

I. Introduction

Catalog Number: M00309

Cell Line Name: 293/TP

Gene Synonyms: TXA2-R; TBXA2R

Expressed Gene: Genbank Accession Number NM_001060; no expressed tags

Host Cell: 293

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

Stability: 16 passages

Application: Functional assay for TP receptor

Freeze Medium: 45% culture medium, 45% FBS, 10% DMSO

Complete Growth Medium: DMEM, 10% FBS

Culture Medium: DMEM, 10% FBS, 300 μg/ml G418

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery.

II. Background

The thromboxane A2 (TxA2) receptor (TP) is a member of the G protein-coupled receptor (GPCR) superfamily which mediates TxA2-induced platelet aggregation and vasoconstriction. Dysregulation of TxA2 synthesis and function has been implicated in the pathogenesis of a number of disease states including myocardial ischemia, asthma, pregnancy-induced hypertension, and a variety of kidney diseases. TP receptors (Thromboxane A2 receptors) are widely distributed among different organ systems and localized on both cell membranes and in intracellular structures. Two isoforms of human TPs have been cloned from placenta (TPα) and endothelium (TPβ) that differ in their mechanisms and kinetics of desensitization and internalization. The TPs are linked via the Gq/G11 class of G proteins to phospholipase C (PLC), which hydrolyzes phosphoinositides to two potent second messengers: inositol 1,4,5-trisphosphate, which leads to an increase in cytoplasmic free calcium, and diacylglycerol (DAG), which activates protein kinase C (PKC).

§: 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 U-46619 in 293/TP and 293 cells

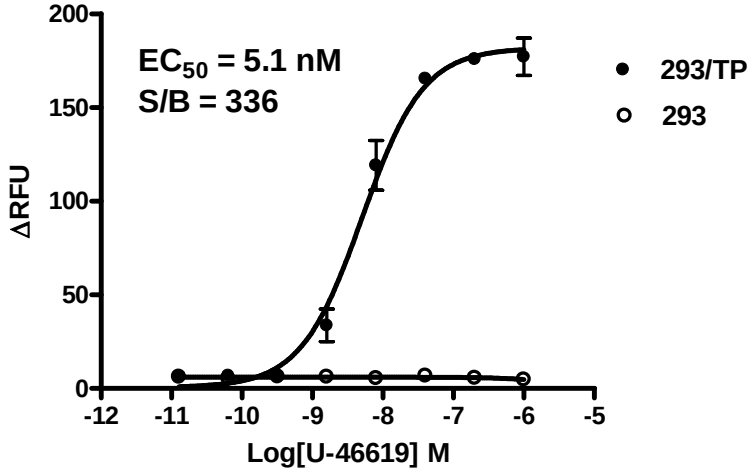

Figure 1. U-46619-induced concentration-dependent stimulation of intracellular calcium mobilization in 293/TP and 293 cells. The cells were loaded with Calcium-4 prior to stimulation with a TP receptor agonist, U-46619. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of U-46619 (Mean ± SD, n = 2). The EC_{50} of U-46619 on TP in 293 cells was 5.1 nM. The S/B of U-46619 on TP in 293 cells was 336.

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 the 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 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 300 µg/ml 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 a 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 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. Hirata, M., Hayashi, Y., *et al*. (1991) Cloning and expression of cDNA for a human thromboxane A2 receptor. *Nature*, 349: 617-620.
2. Nusing, R.M., Hirata, M., *et al*. (1993) Characterization and chromosomal mapping of the human thromboxane A2 receptor gene. *J. Biol. Chem.*, 268: 25253-25259.
3. Brass LF, Shaller CC and Belmonte EJ (1987) Inositol 1,4,5-triphosphate-induced granule secretion in platelets. Evidence that the activation of phospholipase C mediated by platelet thromboxane receptors involves a guanine nucleotide binding protein-dependent mechanism distinct from that of thrombin. *J. Clin. Invest*, 79: 1269–1275.

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