Human Recombinant EP1 Prostanoid Receptor Stable Cell Line

Technical Manual No. TM0413 **Version 10132010**

I. Introduction

Catalog Number: M00228

Cell Line Name: 293/EP1

Gene Synonyms: PTGER1, EP1

Expressed Gene: Genbank Accession Number NM_000955.2; no expressed tags

Host Cell: 293

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

Stability: 16 passages

Application: Functional assay for EP1 receptor

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

Complete Growth Medium: DMEM, 10% FBS

Culture Medium: DMEM, 10% FBS, 200 μg/ml Zeocin, 250 μg/ml G418

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. Background

Prostaglandins are known to affect the nervous system and can modulate synaptic transmission and neurotransmitter release, the sleep/wake cycle, fever, pain, and the immune system. Prostaglandin E2 receptor, EP1 subtype (EP1/PTGER1) is a receptor for prostaglandin E2 (PGE2). The members of the EP receptor family, EP1, EP2, EP3, and EP4, elicit their actions by altering cyclic adenosine monophosphate (cAMP) or intracellular calcium concentrations. EP1 activates phospholipase C and phosphatidylinositol turnover and stimulates the release of intracellular calcium via a Gi/Gq-coupled mechanism. EP2 and EP4 both signal through a Gs-coupled mechanism that stimulates adenylyl cyclase and increases intracellular levels of cAMP. EP1 appears to mediate the effects of PGE2 in promoting the formation of precancerous lesions in animal models of colon cancer. In addition, EP1 has an inhibitory effect on stress-induced aggressive and risk-taking behaviors in mice.

§: 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.

860 Centennial Ave., Piscataway, NJ 08854, USA
Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: info@genscript.com Web: www.genscript.com

III. Representative Data

Concentration-dependent stimulation of intracellular calcium mobilization by PGE2 in 293/EP1 and 293 cells

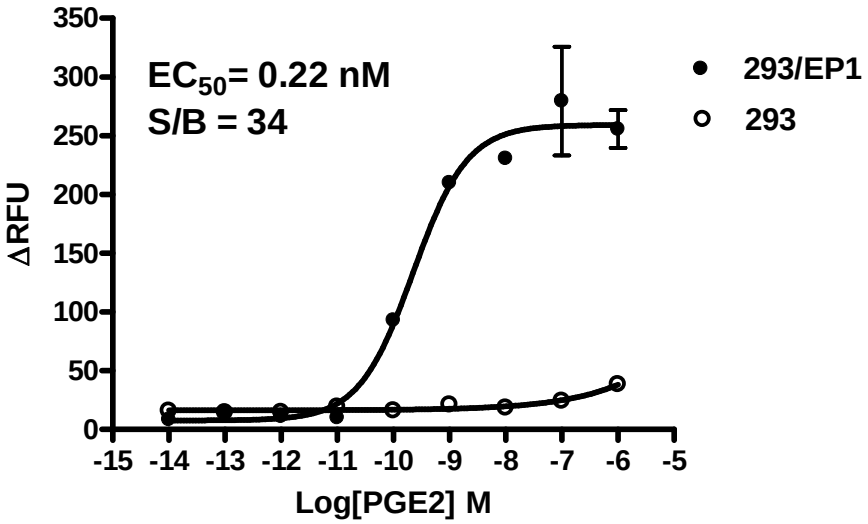

Figure 1. PGE2-induced concentration-dependent stimulation of intracellular calcium mobilization in 293/EP1 and 293 cells. The cells were loaded with Calcium-4 prior to stimulation with an EP1 receptor agonist, PGE2. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of PGE2 (Mean ± SD, n = 2). The EC_{50} of PGE2 on EP1 in 293 cells was 0.22 nM. The S/B of PGE2 on EP1 in 293 cells was 34.

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 Zeocin and G418 to concentrations of 200 µg/ml and 250 µg/ml respectively the following day.

860 Centennial Ave., Piscataway, NJ 08854, USA
Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: info@genscript.com Web: www.genscript.com

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. Narumiya, S., Sugimoto, Y., and Ushikubi, F. (1999). Prostanoid receptors: Structures, properties, and functions. *Physiol. Rev*. 79, 1193-1226.
2. Narumiya, S. (2003). Prostanoids in immunity: Roles revealed by mice deficient in their receptors. *Life Sci.* 74, 391-395.
3. Kaufmann, W. E., Andreasson, K. I., Isakson, P. C., and Worley, P. F. (1997). Cyclooxygenases and the central nervous system. *Prostaglandins*. 54, 601-624.
4. Watanabe, K., Kawamori, T., Nakatsugi, S., Ohta, T., Ohuchida, S., Yamamoto, H., Maruyama, T., Kondo, K., Ushikubi, F., Narumiya, S., et al. (1999). Role of the prostaglandin E receptor subtype EP1 in colon carcinogenesis. *Cancer Res*.59, 5093-5096.
5. Matsuoka Y, Furuyashiki T, Yamada K, Nagai T, Bito H, Tanaka Y, Kitaoka S, Ushikubi F, Nabeshima T, Narumiya S (2005) Prostaglandin E receptor EP1 controls impulsive behavior under stress. *Proc Natl Acad Sci USA* 102:16066–16071

GenScript USA Inc.
860 Centennial Ave., Piscataway, NJ 08854
Tel: 732-885-9188, 732-885-9688
Fax: 732-210-0262, 732-885-5878
Email: info@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 or its components are used for research purposes only.

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 purposes other than research, contact Marketing Department, GenScript USA Inc., 860 Centennial Avenue, Piscataway, New Jersey 08854, U.S.A. Phone: 1-732-885-9188. Fax: 1-732-210-0262. Email: marketing@genscript.com.

860 Centennial Ave., Piscataway, NJ 08854, USA
Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: info@genscript.com Web: www.genscript.com