

Human Recombinant Angiotensin Receptor 1 Stable Cell Line

Technical Manual No TM0623

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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: M00458

Cell Line Name: HEK293/AT1

Gene Synonyms: AGTR1; AG2S; AGTR1A; AGTR1B; AT1; AT1B; AT2R1; AT2R1A; AT2R1B; HAT1R

Expressed Gene: Genbank Accession Number NM_000685; no expressed tags

Host Cell: HEK293

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

Stability: 16 passages

Application: Functional assay for AT1 receptor

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

Complete Growth Medium: DMEM, 10% FBS

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

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. Background

Angiotensin receptor 1 (AT1 receptor) is a member of angiotensin receptors which are responsible for the signal transduction of the main effector hormone. Effects mediated by the AT1 receptor include vasoconstriction, aldosterone synthesis and secretion, increased vasopressin secretion, cardiac hypertrophy, augmentation of peripheral noradrenergic activity, vascular smooth muscle cells proliferation, decreased renal blood flow, renal renin inhibition, renal tubular sodium reuptake, modulation of central sympathetic nervous system activity, cardiac contractility, central osmocontrol and extracellular matrix formation.

§: 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 Angiotensin II in HEK293/AT1 and HEK293 cells

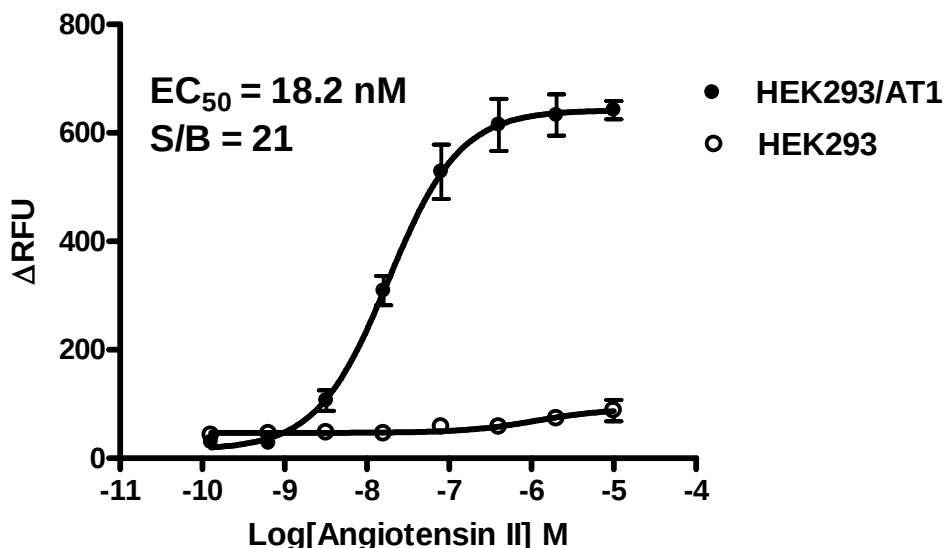


Figure 1. Angiotensin II-induced concentration-dependent stimulation of intracellular calcium mobilization in HEK293/AT1 and HEK293 cells. The cells were loaded with Calcium-4 prior to stimulation with an AT1 receptor agonist, Angiotensin II. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (5-fold dilution) of Angiotensin II (Mean $\pm$ SD, n = 2). The EC_{50} of Angiotensin II on AT1 in HEK293 cells was 18.2 nM. The S/B of Angiotensin II on AT1 in HEK293 cells was 21

Notes:

1. EC_{50} value is calculated with four parameter logistic equation:

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{((\text{Log}EC_{50} - X) * \text{HillSlope})})$$

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.
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 G418 to a concentration of 300 μ g/ml the following day.

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. Catt KJ, Inagami T, Wright JW, *et al.* (2000) International union of pharmacology. XXIII. The angiotensin II receptors. *Pharmacol. Rev.* 52(3): 415–72.
2. Higuchi S, Ohtsu H, Suzuki H, *et al.* (2007) Angiotensin II signal transduction through the AT1 receptor: novel insights into mechanisms and pathophysiology. *Clin. Sci.* 112(8): 417–28.
3. Catt KJ, Mendelsohn FA, Millan MA, *et al.* (1984) The role of angiotensin II receptors in vascular regulation. *J. Cardiovasc. Pharmacol.* 6 Suppl 4:S575-86.

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