

Angiotensin II Recptor-like 1 (AGTRL1) ACTOne™ Stable Cell Line

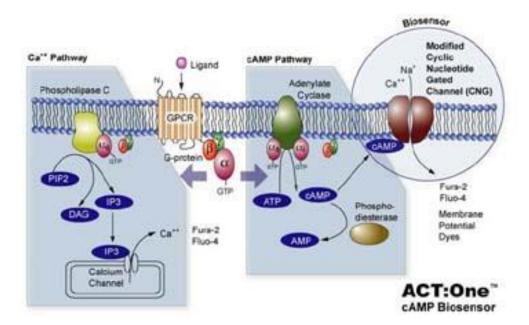
CATALOG NUMBER: CL-11-AGTRL1

Introduction

The angiotensin II receptor-like 1 receptor (AGTRL1), also known as apelin receptor, is a G protein-coupled receptor which binds apelin. AGTRL1 is related to the angiotensin receptor, but is actually an apelin receptor that inhibits adenylate cyclase activity and plays a counter-regulatory role against the pressure action of angiotensin II by exerting hypertensive effect. It functions in the cardiovascular and central nervous systems, in glucose metabolism, in embryonic and tumor angiogenesis and as a human immunodeficiency virus (HIV-1) coreceptor.

Description

Human AGTRL1 ACTOne™ is a HEK-293 CNG cell line that expresses recombinant human AGTRL1. HEK-293 CNG cells express a modified CNG (Cyclic Nucleotide Gated) channel that opens in response to elevated intracellular cAMP levels and consequently result in ion flux (often detectable by calcium-responsive dye, Cat# CA-C155) and cell membrane depolarization which can be easily measured with fluorescent Membrane Potential Dye (Cat# CA-M165). The assay allows both end-point and kinetic measurement of intracellular cAMP changes with a FLIPR, or a fluorescence microplate reader.



Parental Cells

HEK-293 CNG cells (originally developed by BD Biosciences by introducing CNG in HEK-293 cells) (Cat# CL-03-PC20)

Gene/Enzyme Introduced

AGTRL1 (Genbank Accession No. P35414)

Applications

- cAMP dependent assay for Gi-coupled human AGTRL1
- cell based high-throughput screening of human AGTRL1 inhibitors

Functional Test





- this cell line has been tested positive for AGTRL1 specific response
- surviving rate: More than 2.5 million/vial on the second day after thawing
- the receptor specific activity is stable for 10 weeks continuous passage

Mycoplasma Contamination Test

This lot of cells has been tested and found to be free of mycoplasma contamination.

Content

• Stable cells: 1 mL (1 x 10⁶ cells/mL in 70% DMEM, 20% FBS, 10% DMSO)

Growth Properties

Adherent

Cell Culture Medium

- Growth medium: DMEM-10% FBS supplemented with 250 μg/ml G418, 1 μg/ml Puromycin
- Freezing medium: 10% DMSO, 90% complete cell culture medium

Storage

Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

Data Analysis

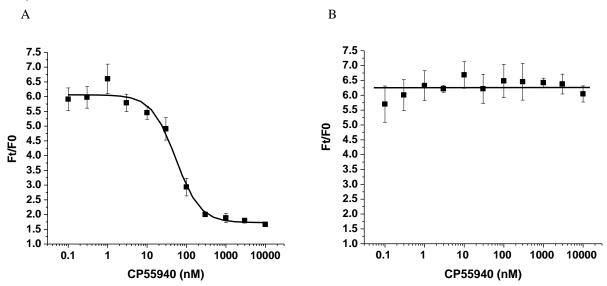


Figure 1. Response of ACTOne™ AGTRL1 cell line & parental cell line to APELIN 13.

ACTOneTM AGTRL1 cells and parental cells (Cat# CL-03-PC20) were plated overnight in 20 μ l culture medium on a 384 well Biocoat plate. The next day, cells were dye-loaded with 20 μ l/well of 1x Dye-loading solution (membrane potential dye kit, Cat# CA-M165). After 2 hours of incubation at room temperature, two readings were obtained prior to and 30 min after the addition of APELIN 13. Ratios of the two readings (F/F0) are plotted in the figure.

- A. Dose response curve of APELIN 13 in ACT One^{TM} AGTRL1 cell line. EC50 = 46 pM in the presence of PDE inhibitor Ro 20-1724 and β -adrenoceptor agonist isoproterenol.
- B. Parental cells do not respond to APELIN 13.

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