Dopamine Receptor D1 (DRD1) ACTOne™ Stable Cell Line

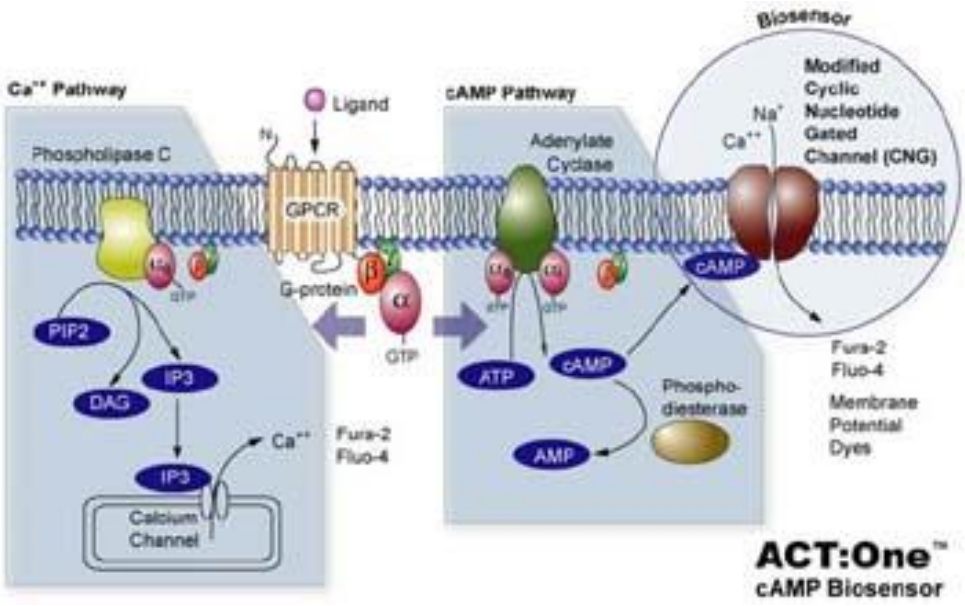
CATALOG NUMBER: CL-01-DRD1

Introduction

The D1 subtype of dopamine receptor (DRD1) is the most abundant dopamine receptor in the central nervous system. This G protein-coupled receptor stimulates adenylate cyclase and indirectly activates cyclic AMP-dependent protein kinases. D1 receptors regulate neuronal growth and development, mediate some behavioral responses, and modulate dopamine receptor D2-mediated events.

Description

Human DRD1 ACTOne™ is a HEK-293 CNG cell line that expresses recombinant human DRD1. 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

DRD1 (Genbank Accession No. NP_000785.1)

Applications

- cAMP dependent human DRD1 cell based assay
- cell based high-throughput screening of human DRD1 inhibitors

Functional Test

- this cell line has been tested positive for DRD1 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 10^6 cells/mL in 70% DMEM, 20% FBS, 10% DMSO)

Growth Property

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

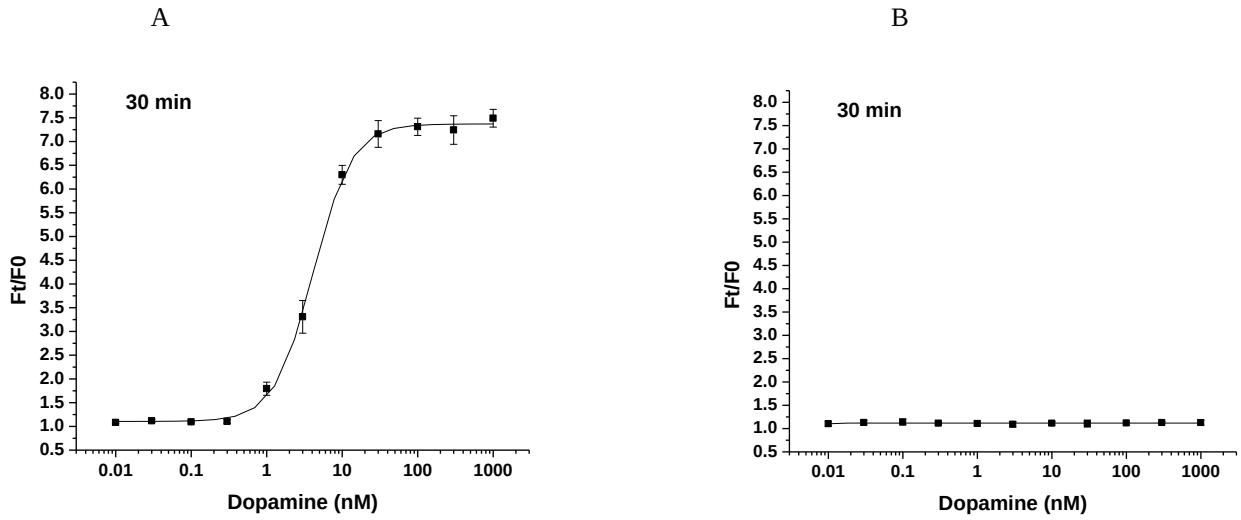


Figure 1. Response of ACT*One*™ DRD1 cell line & parental cell line to dopamine

ACTOne™ DRD1 cells and parental cells (Cat# CL-03-PC20) were plated overnight in 20 µl culture medium on a 384 well Biocoat plate. The next day, cells were dye-loaded with 20 µl/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 [Nle4, D-Phe7]α-MSH. Ratios of the two readings (F/F0) are plotted in the figure.

A. Dose response curve of dopamine in ACT*One*™ DRD1 cell line. EC50 = 4.1 nM in the presence of PDE inhibitor Ro 20-1724.
B. Parental cells do not respond to dopamine.

Notice to Purchaser

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