

## Dopamine Receptor 2 (DRD2) ACTOne™ Stable Cell Line

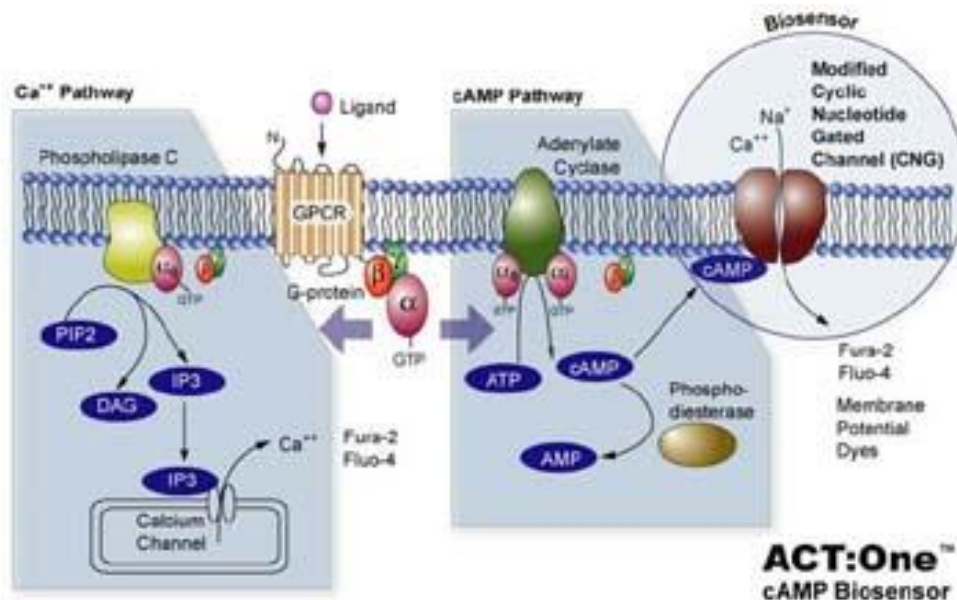
CATALOG NUMBER: CL-11-DRD2

### Introduction

DRD2 is a member of the dopamine receptor G-protein-coupled receptor family that also includes DRD1, DRD3, DRD4 and DRD5. They are located primarily in the caudate putamen, nucleus accumbens and olfactory tubercle where they are involved in the modulation of locomotion, reward, reinforcement and memory and learning.

### Description

Human DRD2 ACTOne™ is a HEK-293 CNG cell line that expresses recombinant human DRD2. 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

DRD2 (Genbank Accession No. NP\_057658.1)

### Applications

- cAMP dependent assay for Gi-coupled human DRD2
- cell based high-throughput screening of human DRD2 inhibitors

### Functional Test

- this cell line has been tested positive for DRD2 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 ( $1 \times 10^6$  cells/mL in 70% DMEM, 20% FBS, 10% DMSO)

## Growth Properties

Adherent

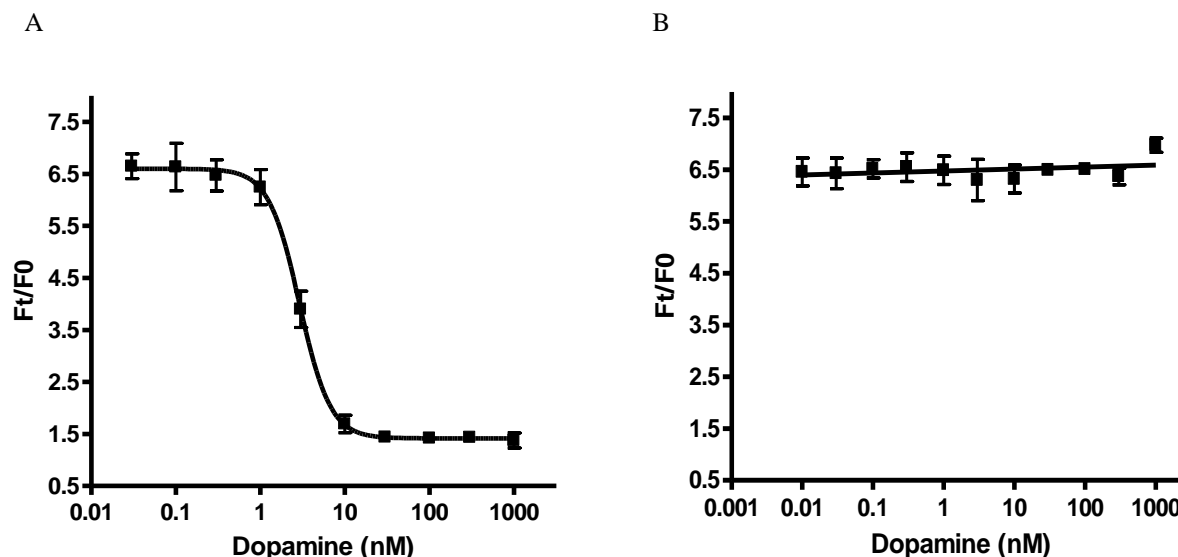
## Cell Culture Medium

- Growth medium: DMEM-10% FBS supplemented with 250  $\mu$ g/ml G418, 1  $\mu$ g/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  $-130^{\circ}\text{C}$ , preferably in liquid nitrogen vapor, until ready for use.

## Data Analysis



**Figure 1. Dose response curve of dopamine in ACTOne™ DRD2 cell line.**

ACTOne™ DRD2 cells and parental cells (Cat# CL-03-PC20) were plated overnight in 20  $\mu$ l culture medium on a 384 well Biocoat plate. The next day, cells were dye-loaded with 20  $\mu$ 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 dopamine. Ratios of the two readings (F/F0) are plotted in the figure.

- Dose response curve of Dopamine in ACTOne™ DRD2 cell line. EC50 = 2.8 nM in the presence of 25  $\mu$ M PDE inhibitor Ro 20-1724 and 300 nM  $\beta$ -adrenoceptor agonist isoproterenol.**
- Parental cells do not respond to Dopamine.**

## Notice to Purchaser

This cell line is sold with a use license. It may not be transferred to third parties, resold, modified for resale without written approval of eEnzyme LLC. Refer to the license agreements for details on the usage restrictions.

