

## Muscarinic Acetylcholine Receptor M4 (CHRM4) ACTOne™ Stable Cell Line

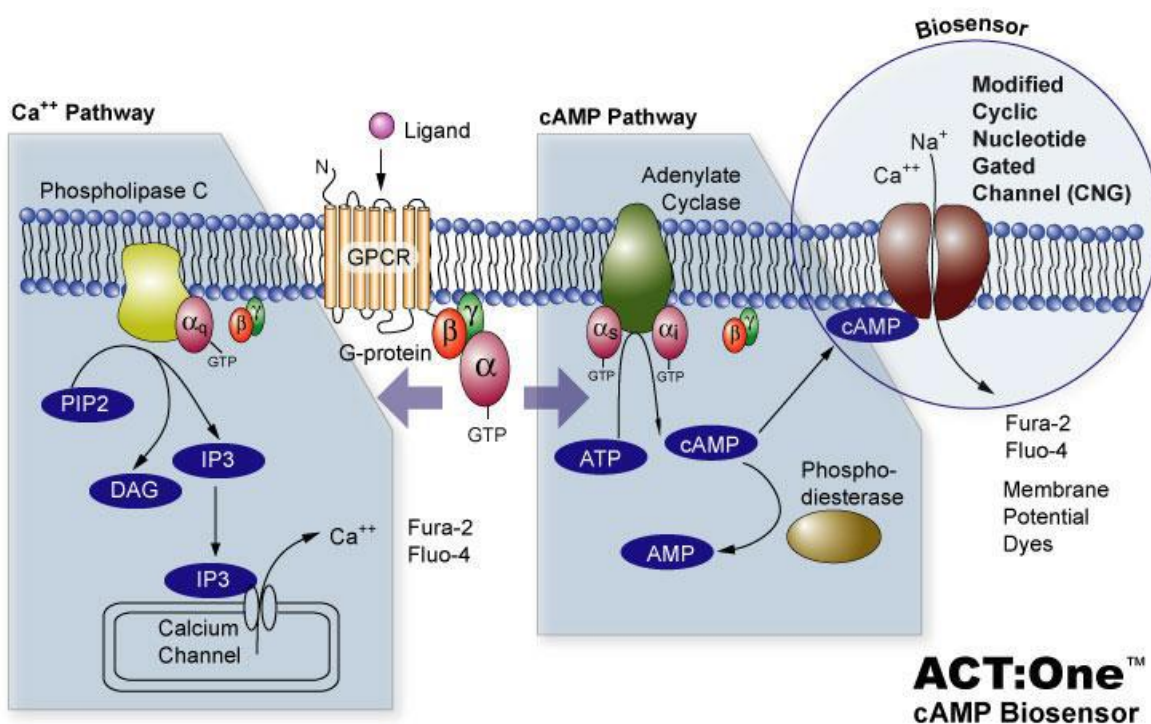
CATALOG NUMBER: CL-01-CHRM4

### Introduction

The muscarinic cholinergic receptors belong to a larger family of G protein-coupled receptors. The functional diversity of these receptors is defined by the binding of acetylcholine and includes cellular responses such as adenylate cyclase inhibition, phosphoinositide degeneration, and potassium channel mediation. M4 receptor-deficient mice exhibit increased locomotor stimulation in response to D1 agonists, amphetamine and cocaine. Neurotransmission in the striatum influences extrapyramidal motor control, thus alterations in M4 activity may contribute to conditions such as Parkinson's Disease.

### Description

Human CHRM4 ACTOne™ is a HEK-293 CNG cell line that expresses recombinant human CHRM4. 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 FDSS, 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

CHRM4 (Genbank Accession No. NP\_000372.2)

### Applications

- cAMP dependent human CHRM4 receptor cell based assay

- cell based high-throughput screening of human CHRM4 receptor agonists/antagonists

#### Functional Test

- this cell line has been tested positive for CHRM4 receptor 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 CHRM4 receptor cells: 1 mL (1 x 10<sup>6</sup> cells/mL in 70% DMEM, 20% FBS, 10% DMSO)

#### Growth Properties

Adherent

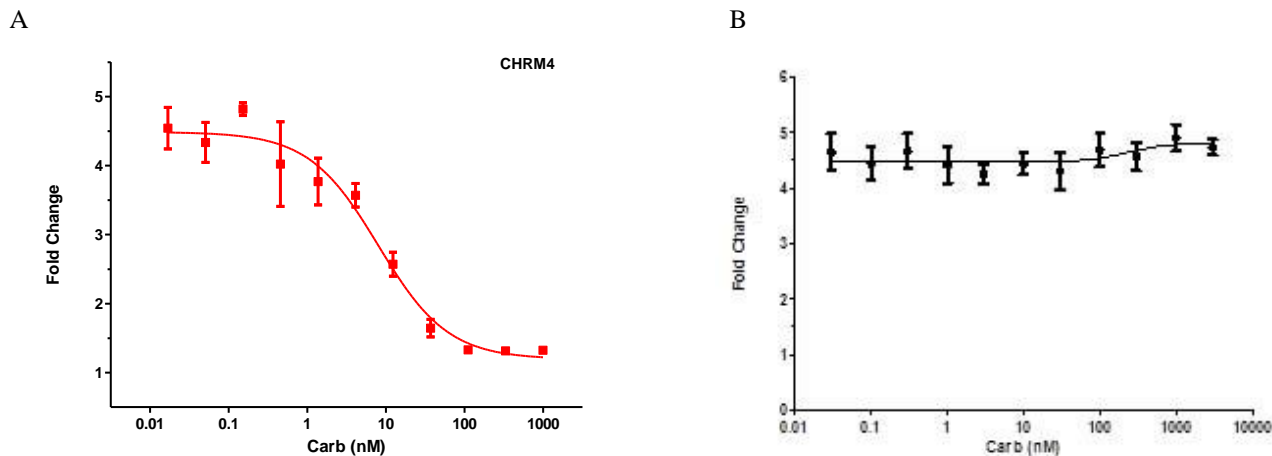
#### Cell Culture Medium

- 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 Example



**Figure 1. Response of ACTOne™ CHRM4 cell line & parental cell line to Carb**

ACTOne™ CHRM4 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 50 min after the addition of Carb. Ratios of the two readings (F/F<sub>0</sub>) are plotted in the figure.

- Dose response curve of Carb in ACTOne™ CHRM4 cell line. EC<sub>50</sub> = 8.1 nM in the presence of PDE inhibitor Ro 20-1724 and β-adrenoceptor agonist isoproterenol.
- Parental cells do not respond to Carb.

### Notice to Purchaser

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