

Chemokine (C-X-C Motif) Receptor 4 (CXCR4) ACTOne™ Stable Cell Line

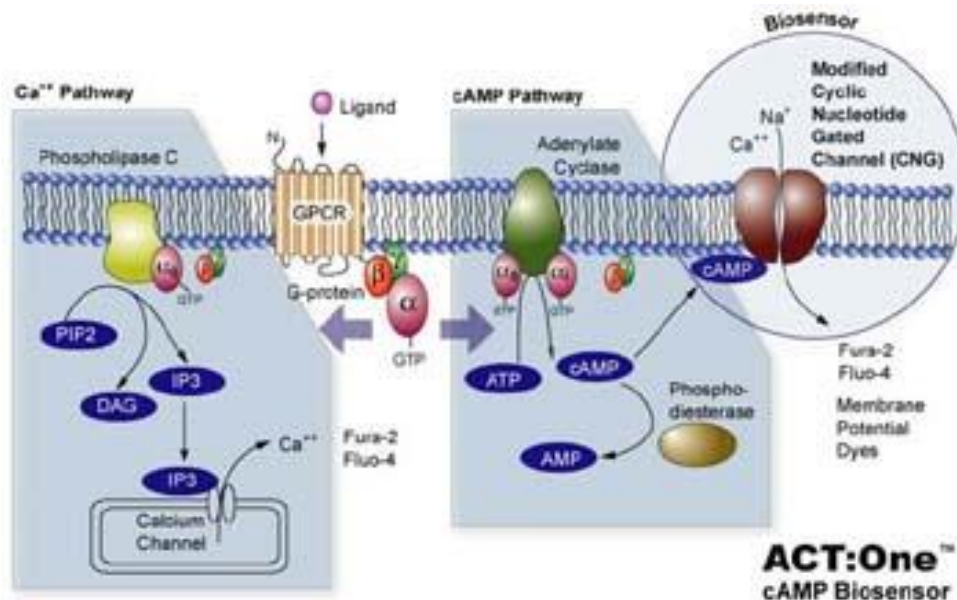
CATALOG NUMBER: CL-11-CXCR4

Introduction

The C-X-C chemokine receptor type 4 (CXCR4) also known as fusin or CD184, is a receptor for the C-X-C chemokine CXCL12/SDF-1 that transduces a signal by increasing intracellular calcium ion levels and enhancing MAPK1/MAPK3 activation. It acts with the CD4 protein to support HIV entry into cells and is also highly expressed in breast cancer cells.

Description

Human CXCR4 ACTOne™ is a HEK-293 CNG cell line that expresses recombinant human CXCR4. 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

CXCR4 (Genbank Accession No. AF052572)

Applications

- cAMP dependent assay for Gi-coupled human CXCR4
- cell based high-throughput screening of human CXCR4 inhibitors

Functional Test

- this cell line has been tested positive for CXCR4 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 (1×10^6 cells/mL in 70% DMEM, 20% FBS, 10% DMSO)

Growth Properties

Adherent

Cell Culture Medium

- Growth medium: DMEM-10% FBS supplemented with 250 μ g/ml G418, 1 μ 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°C , preferably in liquid nitrogen vapor, until ready for use.

Data Analysis

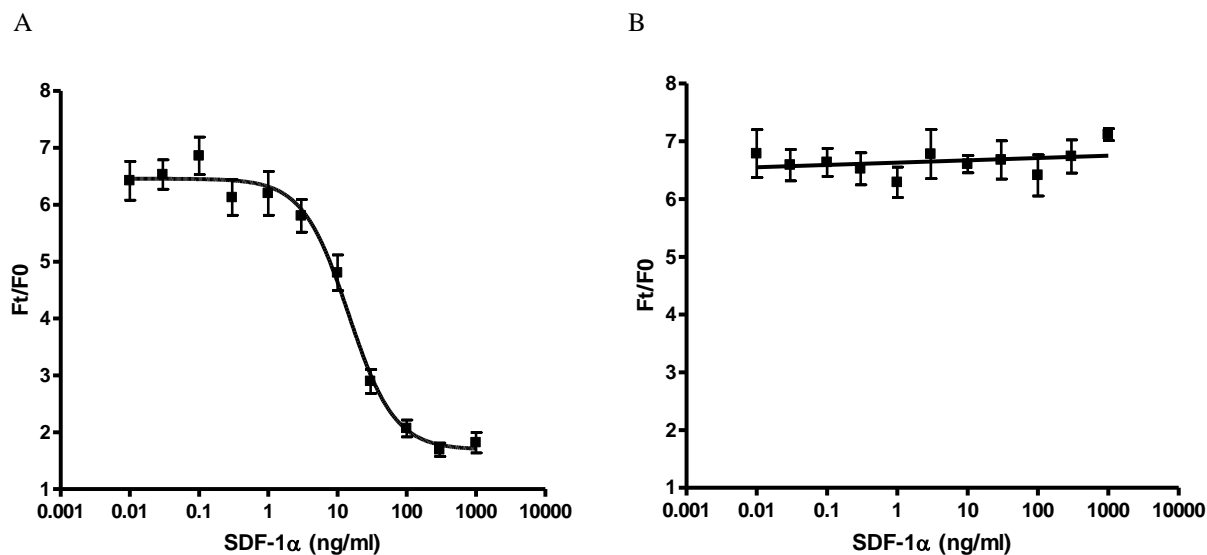


Figure 1. Response of ACTOne™ CXCR4 cell line & parental cell line to SDF-1 α .

ACTOne™ CXCR4 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 SDF-1 α . Ratios of the two readings (F/F0) are plotted in the figure.

- Dose response curve of SDF-1 α in ACTOne™ CXCR4 cell line. EC50 = 14 ng/ml in the presence of PDE inhibitor Ro 20-1724 and β -adrenoceptor agonist isoproterenol.**
- Parental cells do not respond to SDF-1 α .**

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