Human Recombinant CXCR4 Receptor Stable Cell Line

Cat. No. M00556 **Version 03232016**

I. INTRODUCTION

Catalog Number: M00556

Cell Line Name: CHO-K1/CXCR4/$G_{\alpha 15}$

Gene Synonyms: CXCR4; CD184

Expressed Gene: Genbank Accession Number NM_003467; no expressed tags

Host Cell: CHO-K1/$G_{\alpha 15}$

Quantity: Two vials of frozen cells (3×10^6 per vial)

Stability: 16 passages

Application: Functional assay for CXCR4 receptor

Freeze Medium: 45% culture medium, 45% FBS, 10% DMSO

Complete Growth Medium: Ham's F12, 10% FBS

Culture Medium: Ham's F12, 10% FBS, 4 μg/ml puromycin, 100 μg/ml Hygromycin B

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery.

II. BACKGROUND

CXCR4 is a receptor for the C-X-C chemokine SDF-1 (Stromal Cell-Derived Factor 1). It is involved in haematopoiesis and cardiac ventricular septum formation, and plays an essential role in vascularization of the gastrointestinal tract, cerebellar development and survival of hippocampal-neuron of central nerve system. CXCR4 also acts as a primary receptor for some HIV-2 isolates and as a co-receptor with CD4 for HIV-1 X4 viruses.

§: GenScript employs a PCR-based method to test the mycoplasma. The test covers 11 of the most common strains of mycoplasma, (covering approximately 95% of M. fermentans, M. hyorhinis, M. arginini, M. orale, M. salivarium, M. hominis, M. pulmonis, M. arthritidis, M. neurolyticum, M. hyopneumoniae and M. capricolum) and one species Ureaplasma (U. urealyticum), with sufficient sensitivity and specificity.

III. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by h-CXCL12b/SDF-1β in CHO-K1/$G_{\alpha 15}$/CXCR4 and CHO-K1/$G_{\alpha 15}$ cells

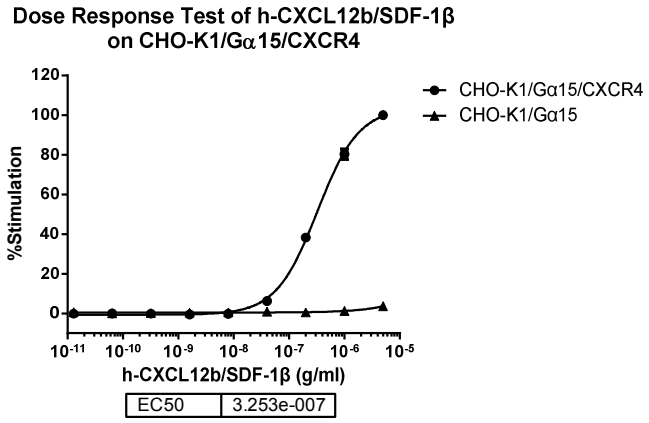

Figure 1. h-CXCL12b/SDF-1β-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/$G_{\alpha 15}$/CXCR4 and CHO-K1/$G_{\alpha 15}$ cells. The cells were loaded with Calcium-4 prior to stimulation with an CXCR4 receptor agonist, h-CXCL12b/SDF-1β. The intracellular calcium change was measured by FLIPR. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses of h-CXCL12b/SDF-1β (Mean ± SD, n = 2). The EC_{50} of h-CXCL12b/SDF-1β on CXCR4 in CHO-K1/$G_{\alpha 15}$ cells was 325 ng/ml.

Notes:

1. EC_{50} value is calculated with four parameter logistic equation:
   Y=Bottom + (Top-Bottom)/(1+10^((LogEC$_{50}$-X)*HillSlope))
   X is the logarithm of concentration. Y is the response
   Y is RFU and starts at Bottom and goes to Top with a sigmoid shape.
2. Signal to background Ratio (S/B) = Top/Bottom

IV. THAWING AND SUBCULTURING

Thawing Protocol

1. Remove the vial from liquid nitrogen tank and thaw cells quickly in a 37°C water-bath.
2. Just before the cells are completely thawed, decontaminate the outside of the vial with 70% ethanol and transfer the cells to a 15 ml centrifuge tube containing 9 ml of complete growth medium.
3. Pellet cells by centrifugation at 200 x g force for 5 min, and remove the medium.
4. Resuspend the cells in complete growth medium.
5. Transfer the cell suspension to a 10 cm dish with 10 ml of complete growth medium.
6. Grow the cells in incubator with 37°C, 5 %CO_2.
7. Add antibiotic in the following day.

Sub-culturing Protocol

1. Remove the culture medium from cells.
2. Wash cells with PBS (pH=7.4) to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 ml of 0.05% (w/v) Trypsin- EDTA (GIBCO, Cat No. 25300) solution into 10 cm dish and observe the cells under an inverted microscope until cell layer is dispersed (usually within 3 to 5 minutes).
   Note: To avoid cells clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells detach. If cells are difficult to detach, please place the dish in 37°C incubator for ~2 min.
4. Add 6.0 to 8.0 ml of complete growth medium into dish and aspirate cells by gently pipetting.
5. Centrifuge the cells at 200 x g force for 5min, and remove the medium.
6. Resuspend the cells in culture medium and add the cells suspension to new culture dish.
7. Grow the cells in incubator with 37°C, 5 %CO_2.

Subcultivation Ratio: 1:3 to 1:8 weekly.
Medium Renewal: Every 2 to 3 days

V. REFERENCES

1. Hernandez et al. (2003) Mutations in the chemokine receptor gene CXCR4 are associated with WHIM syndrome, a combined immunodeficiency disease. *Nat Genet* 34:70-74.
2. Juarez et al. (2004) Chemokines and their receptors as therapeutic targets: the role of the SDF-1/CXCR4 axis. *Curr Pharm Des* 10:1245-1259.

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