Human chemokine (C-X-C motif) receptor 1 (CXCR1) Stable Cell Line
Cat. No. M00524 **Version 07312020**

I. Product Information

Catalog Number: M00524
Cell Line Name: CHO-K1/CXCR1/Gα15
Aliases: C-C; CD128; CD181; CKR-1; IL8R1; IL8RA; CMKAR1; IL8RBA; CDw128a; C-C-CKR-1
GenBank Accession Number: NM_000634.2 (no expressed tags)
Host Cell line: CHO-K1/Gα15
Culture Properties: Adherent
Quantity: Two vials of frozen cells (>1×10^6 per vial)
Stability: Stable in culture for minimum of 20 passages
Application: Functional assay for CXCR1 receptor (Calcium flux assay)
Freeze Medium: 45% culture medium, 45% FBS (Cat. #10099-141, Gibco), 10% DMSO (Cat. #D2650, Sigma)
Propagation Medium: Ham's F-12K (Kaighn's) (Cat. #21127, Life Technologies), 10% FBS, 3 µg/ml puromycin (Cat. #A11138-03, Gibco), 100 µg/ml Hygromycin B (Cat. #10687010, Invitrogen)
Mycoplasma Status: Negative*
Storage: Liquid nitrogen immediately upon receipt

II. Background

Chemokine (C-X-C motif) receptor 1 (CXCR1) is a rhodopsin-like G protein-coupled receptor. It is one of two high-affinity receptors for the CXC chemokine interleukin-8 (IL-8), a major mediator of immune and inflammatory responses. The structure of human CXCR1 in a lipid bilayer should help to facilitate the discovery of new compounds that interact with GPCRs. GenScript's human CXCR1-expressing stable subline is guaranteed to function properly in calcium flux assay.

** The mycoplasma test was performed with MycoAlert™ PLUS Mycoplasma Detection Kit of Lonza.*

III. Application: Functional assay

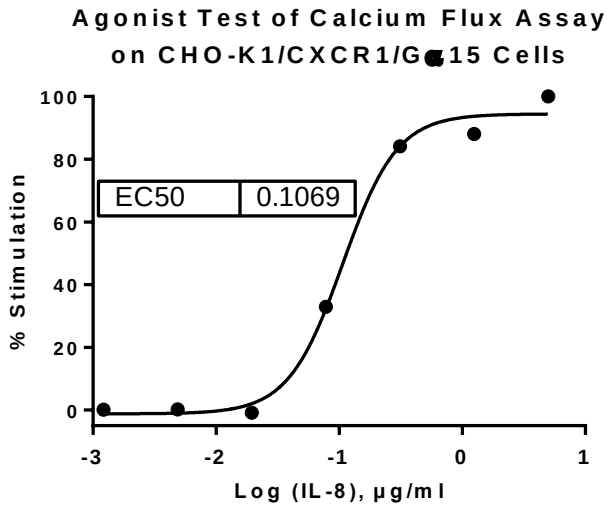

Figure IL-8 induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/CXCR1/Gα15 cells. The cells were loaded with Calcium-4 prior to being stimulated with agonist IL-8. The intracellular calcium change was normalized and measured by FLIPR. The relative fluorescent units (RFU) were normalized and plotted against the log of the cumulative doses of IL-8 (Mean ± SD, n = 2). The EC_{50} of IL-8 on this cell was 0.11 μg/ml.

IV. Thawing and Subculturing

Protocol for recovering stable cell line

1. Prewarm culture medium (Ham's F12 supplemented with 10% FBS) in a 37°C water bath.
2. Remove frozen vial of cells from liquid nitrogen freezer and thaw the cells by gentle agitation in a 37°C water bath until ice crystals disappear.
3. Remove the vial from the water bath and decontaminate it by a briefly spray of 70% ethanol.
4. Unscrew the top of the vial and transfer the cells to a sterile centrifuge tube containing 9 ml complete growth medium.
5. After centrifugation at 125x*g* for 10 minutes at room temperature, discard the supernatant without disturbing the soft pellet. Resuspend the cells in antibiotic-free growth medium. Pipette gently to loosen the pellet and break apart clumps.
6. Transfer the cell suspension into antibiotic-free medium in the culture vessel and mix thoroughly. Recover cells at 37°C, 5% CO_2 overnight.
7. Replace the culture medium with medium that contains 3 μg/ml of puromycin and 100 μg/ml of hygromycin B to maintain selection pressure.

Protocol for subculturing stable cell line

1. Prewarm medium to 37°C in a water bath.
2. Wash cells with PBS buffer to remove all traces of serum.
3. Add 2.0 ml of 0.05% (w/v) Trypsin- EDTA 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 for 5min, and remove the medium.
6. Resuspend the cells in culture medium and aliquot the cells suspension into new culture dishes.
7. Grow the cells in incubator at 37°C with 5 % CO_2.

V. References

1. Busch-Petersen J: Small molecule antagonists of the CXCR2 and CXCR1 chemokine receptors as therapeutic agents for the treatment of inflammatory diseases. Current topics in medicinal chemistry 2006; 6:1345-1352.
2. Park SH, Das BB, Casagrande F, et al: Structure of the chemokine receptor CXCR1 in phospholipid bilayers. Nature 2012; 491:779-783.

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