Human chemokine (C-X-C motif) receptor 3 (CXCR3) Stable Cell Line
Cat. No. M00525 **Version 07312020**

I. Product Information

Catalog Number: M00525
Cell Line Name: CHO-K1/Gα15/CXCR3
Aliases: GPR9; MigR; CD182; CD183; Mig-R; CKR-L2; CMKAR3; IP10-R
GenBank Accession Number: NM_001504.1 (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 CXCR3 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 receiving

II. Background

Chemokine (C-X-C motif) receptor 3 (CXCR3) belongs to the CXC chemokine receptor family. Similar to other chemokine receptors, it is a G protein-coupled receptor (GPCR). CXCR3 has two distinct splice variants, CXCR3A and CXCR3B, demonstrating different binding affinities. CXCR3-A binds to the CXC chemokines CXCL9 (MIG), CXCL10 (IP-10), and CXCL11 (I-TAC) while CXCR3-B can also bind to CXCL4 in addition to CXCL9, CXCL10, and CXCL11. GenScript's human CXCR3A-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

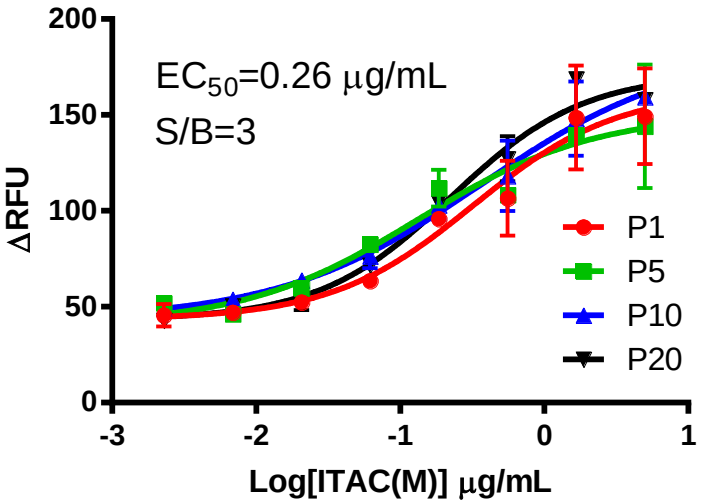

Figure ITAC-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/Gα15/CXCR3 cells. The cells were loaded with Calcium-4 prior to being stimulated with agonist ITAC. The intracellular calcium change was normalized and measured by FLIPR. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses of ITAC (Mean ± SD, n = 2). The EC_{50} of ITAC on this cell was 0.26 μg/ml. P1, P5, P10 and P20 represent the cell passage number.

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. Clark-Lewis I, Mattioli I, Gong J-H, and Loetscher P: Structure-function relationship between the human chemokine receptor CXCR3 and its ligands. Journal of Biological Chemistry 278: 289-295, 2003.
2. Lasagni L, Francalanci M, Annunziato F, Lazzeri E, Giannini S, Cosmi L, Sagrinati C, Mazzinghi B, Orlando C, and Maggi E: An alternatively spliced variant of CXCR3 mediates the inhibition of endothelial cell growth induced by IP-10, Mig, and I-TAC, and acts as functional receptor for platelet factor 4. The Journal of experimental medicine 197: 1537-1549, 2003.

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