
Human chemokine (C-X-C motif) receptor 3 (CXCR3A) Stable Cell Line

Cat. No.: M00525

Version 06122014

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I. Product Information

Catalog Number: M00525

Cell Line Name: CHO-K1/human CXCR3A Gα15/

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

Quantity: Two vials of frozen cells (3×10⁶ per vial)

Stability: Stable in culture over a minimum of 20 passages

Application: Functional assay for CXCR3 receptor

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

Propagation Medium: Ham's F12, 10% FBS, 3 µg/ml puromycin, 100 µg/ml Hygromycin B

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 (ITAC) 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.

[§]: 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. Application: Functional assay

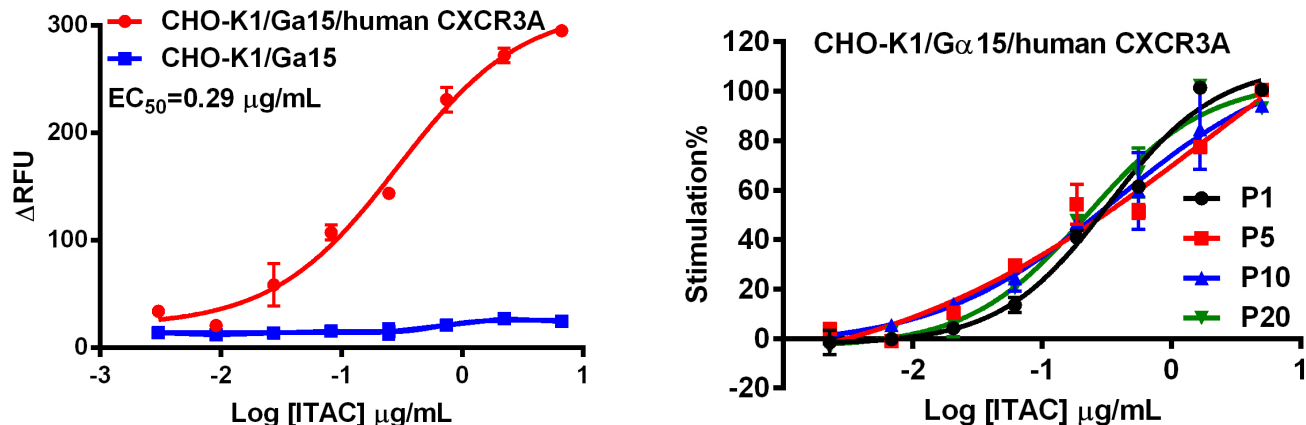


Figure Concentration dependent stimulation of intracellular calcium mobilization in CHO-K1/Gα15/human CXCR3A cells upon treatment with its ligand human ITAC.

The human CXCR3A-expressing stable subline (GenScript, Cat No.: M00525) was loaded with Calcium-4 prior to stimulation with a CXCR3A receptor agonist, human ITAC (GenScript, Cat No.: Z03246). The intracellular calcium mobilization was monitored by FLIPR® Tetra. The relative fluorescent units (RFU) were plotted against the cumulative concentrations of human ITAC (Mean ± SD, n = 2). The EC₅₀ value of human ITAC stimulation of calcium mobilization on human CXCR3A receptor was 0.29 μg/mL (Left panel). The human CXCR3A expression stability was evaluated by the intracellular calcium mobilization assay on CHO-K1/Gα15/human CXCR3A cells cultured up to Passage 20 (Right panel). The RFU of each passage was normalized to the RFU of Passage 1 at different human ITAC concentrations. The CHO-K1/Gα15/human CXCR3A is stable in culture for a minimum of 20 passages.

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 125xg 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% CO₂ 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 % CO₂.

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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