Human chemokine (C-C motif) receptor 1 (CCR1) Stable Cell Line

Cat. No.: M00522 Version 06122014

I. Product Information

Catalog Number: M00522
Cell Line Name: CHO-K1/human CCR1/Gα15
Aliases: CD191; CKR-1; CKR1; CMKBR1; HM145; MIP1aR; SCYAR1
GenBank Accession Number: NM_001295 (no expressed tags)
Host Cell line: CHO-K1/Gα15
Quantity: Two vials of frozen cells (3×10^6 per vial)
Stability: Stable in culture over a minimum of 20 passages
Application: Functional assay for CCR1 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

C-C chemokine receptor type 1 (CCR1) is a G protein-coupled receptor member of the CC chemokine subfamily of receptors. Following interaction with its specific chemokine ligands, like macrophage inflammatory protein 1 alpha (MIP-1 alpha), regulated on activation normal T expressed and secreted protein (RANTES), and monocyte chemoattractant protein 3 (MCP-3), it triggers the onset of a process known as chemotaxis. GenScript's human CCR1-expressing stable subline is guaranteed to function properly in the 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

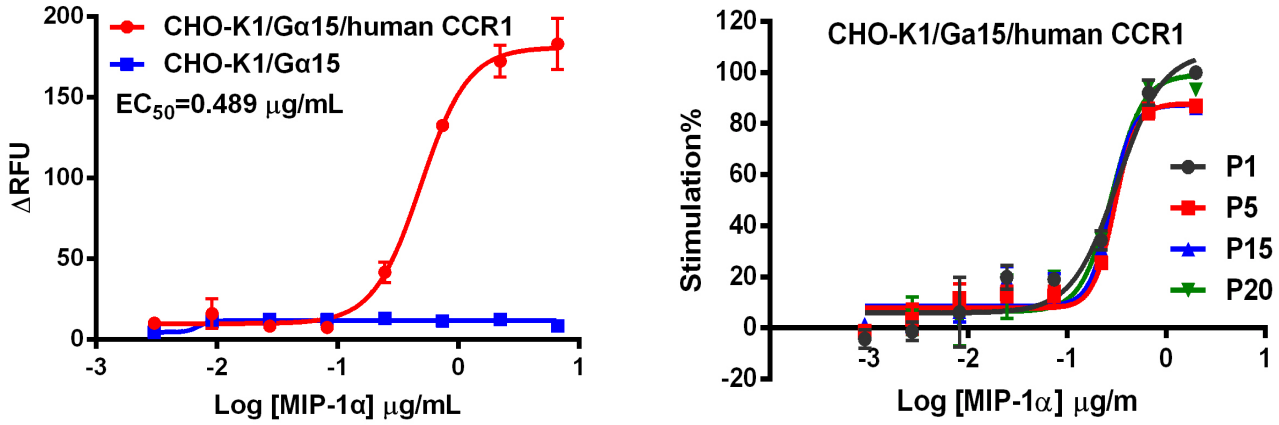

Figure 1 Concentration dependent stimulation of intracellular calcium mobilization in CHO-K1/Gα15/human CCR1 cells upon treatment with its ligand, human MIP-1α.

The human CCR1-expressing stable subline (GenScript, Cat No.: M00522) was loaded with Calcium-4 prior to the stimulation with a human CCR1 receptor agonist, human MIP-1α (GenScript, Cat No.: Z03137). The intracellular calcium mobilization was monitored by FLIPR® Tetra. The relative fluorescent units (RFU) were plotted against the cumulative concentrations of human MIP-1α (Mean ± SD, n = 2). The EC_{50} value of human MIP-1α stimulation of calcium mobilization on human CCR1 receptor was 0.489 μg/mL (Left panel). The human CCR1 expression stability was evaluated by the intracellular calcium mobilization assay on CHO-K1/Gα15/human CCR1 cells cultured up to Passage 20 (Right panel). The RFU of each passage was normalized to the RFU of Passage 1 at different human MIP-1α concentrations. The CHO-K1/Gα15/human CCR1 is stable in culture over 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 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 5 min, 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. Chou CC, Hipkin RW *et.al* (2002) Pharmacological characterization of the chemokine receptor, hCCR1 in a stable transfectant and differentiated HL-60 cells: antagonism of hCCR1 activation by MIP-1beta. *Br J Pharmacol*, 137: 663-675.
2. Combadiere C, Murphy PM *et.al* (1995) Monocyte chemoattractant protein-3 is a functional ligand for CC chemokine receptors 1 and 2B. *J Biol Chem*, 270: 29671-29675.

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