Human chemokine (C-C motif) receptor 5 (CCR5) Stable Cell Line

Cat. No.: M00523 Version 06122014

I. Product Information

Catalog Number: M00523
Cell Line Name: CHO-K1/human CCR5/Gα15
Aliases: CKR5; CCR-5; CD195; CKR-5; CCCKR5; CMKBR5; IDDM22; CC-CKR-5
GenBank Accession Number: NM_000579.3 (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 CCR5 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 5 (CCR5) is a G protein-coupled receptor and a co-receptor for the entry of human immunodeficiency virus-1 (HIV-1) into cells. CCR5 chemokine receptor is involved in leucocytes chemotaxis to sites of inflammation and plays an important role in the macrophages, T cells, and monocytes recruitment. The chemokine ligands that bind to CCR5 are regulated on activation, normal T Cell expressed and secreted (RANTES) and macrophage inflammatory protein 1 alpha (MIP1α). GenScript's human CCR5-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

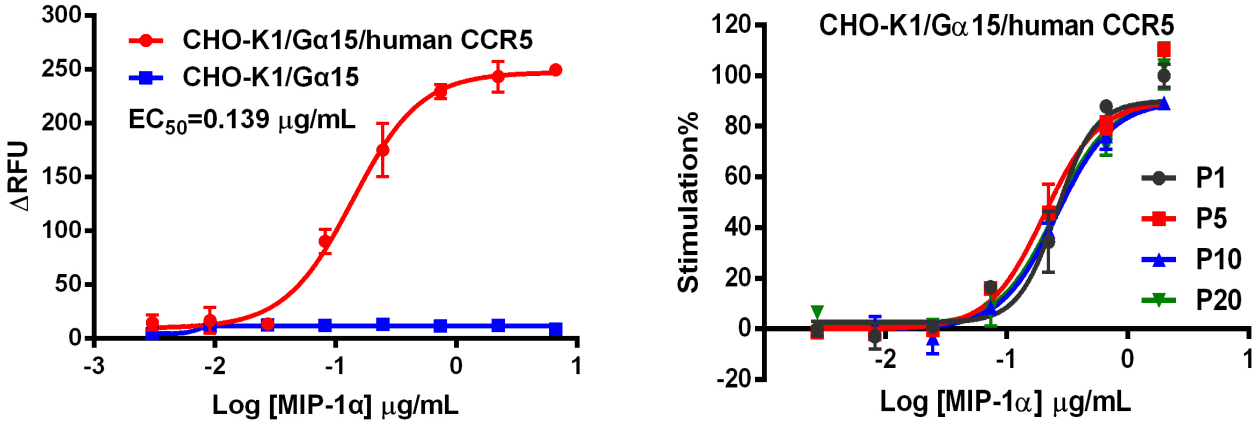

Figure Concentration dependent stimulation of intracellular calcium mobilization in CHO-K1/Gα15/human CCR5 cells upon treatment with its ligand human MIP-1α.

The human CCR5-expressing stable subline (GenScript, Cat No.: M00523) was loaded with Calcium-4 prior to the stimulation with a human CCR5 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-1 stimulation of calcium mobilization on human CCR5 receptor was 0.139 μg/mL (Left panel). The human CCR5 expression stability was evaluated by the intracellular calcium mobilization assay on CHO-K1/Gα15/human CCR5 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 CCR5 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 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. Sanchooli J, Sanadgol N, Kazemi Arababadi M, et al: CCR5 plays important roles in hepatitis B infection. Viral immunology 2014; 27:2-6
2. Maeda K, Das D, Nakata H, et al: CCR5 inhibitors: emergence, success, and challenges. Expert opinion on emerging drugs 2012; 17:135-145
3. Gilliam BL, Riedel DJ,Redfield RR: Clinical use of CCR5 inhibitors in HIV and beyond. Journal of translational medicine 2011; 9 Suppl 1:S9

GenScript USA Inc.
860 Centennial Ave., Piscataway, NJ 08854
Tel: 732-885-9188, 732-885-9688
Fax: 732-210-0262, 732-885-5878
Email: product@genscript.com
Web: http://www.genscript.com

For Research Use Only

Limited Use License Agreement

This is a legal agreement between you (Licensee) and GenScript USA Inc. governing use of GenScript's stable cell line products and protocols provided to licensee. By purchasing and using the stable cell line, the buyer agrees to comply with the following terms and conditions of this label license and recognizes and agrees to such restrictions:

1) The products are not transferable and will be used at the site where they were purchased. Transfer to another site owned by buyer will be permitted only upon written request by buyer followed by subsequent written approval by GenScript.
2) The purchaser cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party.
3) The products sold by GenScript are for laboratory and animal research purposes only. The products are not to be used on humans, for consumption, or for any unlawful uses.

GenScript USA Inc. will not assert against the buyer a claim of infringement of patents owned or controlled by GenScript USA Inc. and claiming this product based upon the manufacture, use or sale of a clinical diagnostic, therapeutic and vaccine, or prophylactic product developed in research by the buyer in which this product or its components has been employed, provided that neither this product nor any of its components was used in the manufacture of such product. For information on the use of this product for other purposes, contact Marketing Department, GenScript USA Inc., 860 Centennial Avenue, Piscataway, New Jersey 08840, U.S.A. Phone: 1-732-885-9188. Fax: 1-732-210-0262. Email: marketing@genscript.com.