
Human chemokine (C-X-C motif) receptor 5 (CXCR5) Stable Cell Line

Cat. No.: M00526

Version 06122014

I	Product Information.....	1
II	Background.....	1
III	Application.....	2
IV	Thawing and Subculturing.....	2
V	References.....	3
	Limited Use License Agreement.....	4

I. Product Information

Catalog Number: M00526

Cell Line Name: CHO-K1/human CXCR5/G α 15

Aliases: BLR1; CD185; MDR15

GenBank Accession Number: NM_001716.4 (no expressed tags)

Host Cell line: CHO-K1/G α 15Quantity: Two vials of frozen cells (3 \times 10⁶ per vial)

Stability: Stable in culture for a minimum of 20 passages

Application: Functional assay for CXCR5 receptor

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

Propagation Medium: Ham's F12, 10% FBS, 3 μ g/ml puromycin, 100 μ g/ml Hygromycin BMycoplasma Status[§]: Negative

Storage: Liquid nitrogen immediately upon receiving

II. Background

Chemokine (C-X-C motif) receptor 5 (CXCR5) belongs to the CXC chemokine receptor family. CXCR5 plays crucial roles in B-cells migration into B-cell follicles of spleen and Peyer patches. CXCR5 reduces maintenance of immature neural cell populations and enhances proliferation of subgranular zone cells in the hippocampal dentate gyrus. 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. hyorhinitis*, *M. arginini*, *M. orale*, *M. salivarium*, *M. hominis*, *M. pulmonis*, *M. arthritis*, *M. neurolyticum*, *M. hyopneumoniae* and *M. capricolum*) and one species *Ureaplasma* (*U. urealyticum*), with sufficient sensitivity and specificity.

III. Application: Functional assay

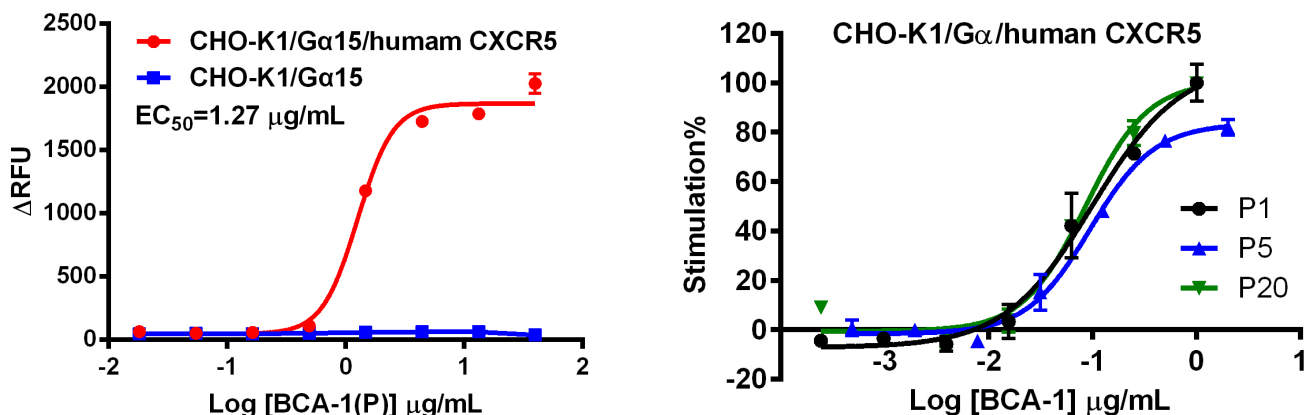


Figure Concentration dependent stimulation of intracellular calcium mobilization in CHO-K1/G α 15/human CXCR5 cells upon treatment with its ligand human B-cell attracting chemokine 1 (BCA-1).

The human CXCR5-expressing stable subline (GenScript, Cat No.: M00526) was loaded with Calcium-4 prior to the stimulation with a human CXCR5 receptor agonist, human BCA-1 (GenScript, Cat No.: Z02826). The intracellular calcium mobilization was monitored by FLIPR® Tetra. The relative fluorescent units (RFU) were plotted against the cumulative concentrations of human BCA-1 (Mean \pm SD, n = 2). The EC₅₀ value of human BCA-1 stimulation of calcium mobilization on human CXCR5 receptor was 1.27 μ g/mL (Left panel). The human CXCR5 expression stability was evaluated by the intracellular calcium mobilization assay on CHO-K1/G α 15/human CXCR5 cells cultured up to Passage 20 (Right panel). The RFU of each passage was normalized to the RFU of Passage 1 at different human BCA-1 concentrations. The CHO-K1/G α 15/human CXCR5 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 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 μ g/ml of puromycin and 100 μ g/ml of hygromycin B to maintain selection pressure.

Protocol for subculturing stable cell line

860 Centennial Ave., Piscataway, NJ 08854, USA

Toll-Free: 1-877-436-7274 Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: product@genscript.com Web: www.genscript.com

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. Stuart MJ, Corrigan F, Baune BT: Knockout of CXCR5 increases the population of immature neural cells and decreases proliferation in the hippocampal dentate gyrus. Journal of neuroinflammation 2014; 11:31
2. Forster R, Mattis AE, Kremmer E, et al: A putative chemokine receptor, BLR1, directs B cell migration to defined lymphoid organs and specific anatomic compartments of the spleen. Cell 1996; 87:1037-1047

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.comWeb: <http://www.genscript.com>**For Research Use Only**

860 Centennial Ave., Piscataway, NJ 08854, USA

Toll-Free: 1-877-436-7274 Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: product@genscript.com Web: www.genscript.com

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.