

---

**Human chemokine (C-X-C motif) receptor 1 (CXCR1) Stable Cell Line**

Cat. No.: M00524

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: M00524

Cell Line Name: CHO-K1/human CXCR1/Gα15

Aliases: C-C; CD128; CD181; CKR-1; IL8R1; IL8RA; CMKAR1; IL8RBA; CDw128a; C-C-CKR-1

GenBank Accession Number: NM\_000634.2 (no expressed tags)

Host Cell line: CHO-K1/Gα15

Quantity: Two vials of frozen cells (3×10<sup>6</sup> per vial)

Stability: Stable in culture over a minimum of 20 passages

Application: Functional assay for CXCR1 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<sup>§</sup>: Negative

Storage: Liquid nitrogen immediately upon receiving

**II. Background**

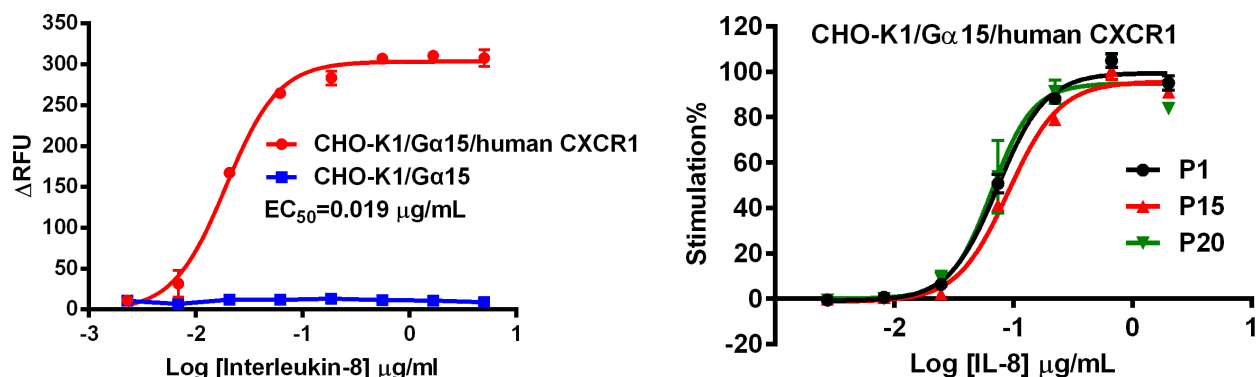
Chemokine (C-X-C motif) receptor 1 (CXCR1) is a rhodopsin-like G protein-coupled receptor. It is one of two high-affinity receptors for the CXC chemokine interleukin-8 (IL-8), a major mediator of immune and inflammatory responses. The structure of human CXCR1 in a lipid bilayer should help to facilitate the discovery of new compounds that interact with GPCRs. GenScript's human CXCR1-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.

---

**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](mailto:product@genscript.com) Web: [www.genscript.com](http://www.genscript.com)

### III. Application: Functional assay



**Figure** Concentration dependent stimulation of intracellular calcium mobilization in CHO-K1/G $\alpha$ 15/human CXCR1 cells upon treatment with its ligand human IL-8.

The human CXCR1-expressing stable subline (GenScript, Cat No.: M00524) was loaded with Calcium-4 prior to the stimulation with a human CXCR1 receptor agonist, human IL-8 (GenScript, Cat No.: Z0306). The intracellular calcium mobilization was monitored by FLIPR® Tetra. The relative fluorescent units (RFU) were plotted against the cumulative concentrations of human IL-8 (Mean  $\pm$  SD,  $n = 2$ ). The  $\text{EC}_{50}$  value of human IL-8 stimulation of calcium mobilization on human CXCR1 receptor was  $0.019 \mu\text{g/mL}$  (Left panel). The human CXCR1 expression stability was evaluated by the intracellular calcium mobilization assay on CHO-K1/G $\alpha$ 15/human CXCR1 cells cultured up to Passage 20 (Right panel). The RFU of each passage was normalized to the RFU of Passage 1 at different human IL-8 concentrations. The CHO-K1/G $\alpha$ 15/human CXCR1 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  $37^{\circ}\text{C}$  water bath.
2. Remove frozen vial of cells from liquid nitrogen freezer and thaw the cells by gentle agitation in a  $37^{\circ}\text{C}$  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  $125\times g$  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  $37^{\circ}\text{C}$ , 5%  $\text{CO}_2$  overnight.
7. Replace the culture medium with medium that contains  $3 \mu\text{g/mL}$  of puromycin and  $100 \mu\text{g/mL}$  of hygromycin B to maintain selection pressure.

#### Protocol for subculturing stable cell line

1. Prewarm medium to  $37^{\circ}\text{C}$  in a water bath.

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](mailto:product@genscript.com) Web: [www.genscript.com](http://www.genscript.com)

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<sub>2</sub>.

#### V. References

1. Busch-Petersen J: Small molecule antagonists of the CXCR2 and CXCR1 chemokine receptors as therapeutic agents for the treatment of inflammatory diseases. Current topics in medicinal chemistry 2006; 6:1345-1352.
2. Park SH, Das BB, Casagrande F, et al: Structure of the chemokine receptor CXCR1 in phospholipid bilayers. Nature 2012; 491:779-783.

#### 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](mailto:product@genscript.com)

Web: <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](mailto:product@genscript.com) Web: [www.genscript.com](http://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](mailto:marketing@genscript.com).