

Human Recombinant Complement Component 5A Receptor 1 Stable Cell Line**Cat. No. M00327****Version 05262014**

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I. INTRODUCTION

Catalog Number: M00327

Cell Line Name: CHO-K1/C5a/Gα15

Gene Synonyms: C5AR1; C5A; C5AR; C5R1; CD88

Expressed Gene: Genbank Accession Number NM_001736; no expressed tags

Host Cell: CHO-K1/Gα15

Quantity: Two vials of frozen cells (3×10^6 per vial)

Stability: 16 passages

Application: Functional assay for C5a receptor

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

Complete Growth Medium: Ham's F12, 10% FBS

Culture Medium: Ham's F12, 10% FBS, 100 µg/ml Hygromycin B, 400 µg/ml G418

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

The complement component 5a receptor 1 (C5a) is $G_{q/11}$ and $G_{i/o}$ -coupled GPCRs. C5a is a significant pathogenic driver in a number of immuno-inflammatory diseases, making C5a inhibition an attractive therapeutic strategy. C5a receptor plays an important role in mediating acute kidney allograft rejection.

§: 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. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by C5a (65-74) in CHO-K1/C5a/Gα15 and CHO-K1/Gα15 cells

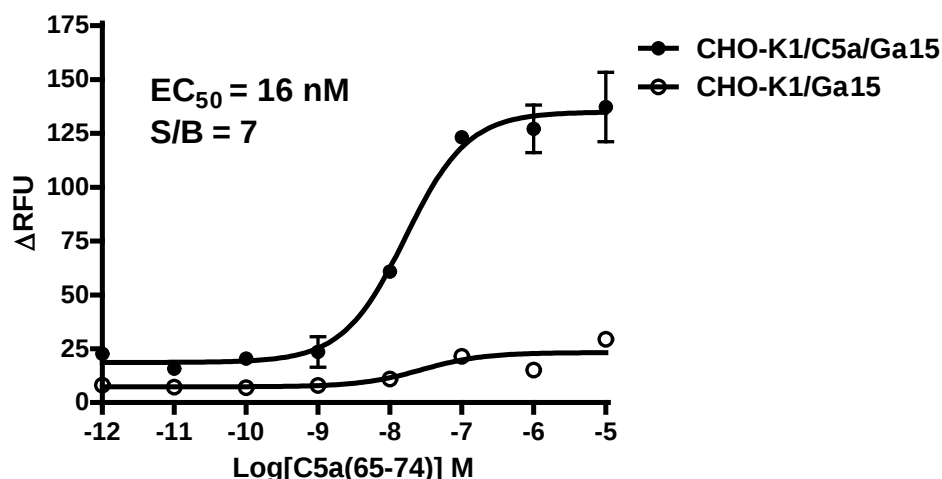


Figure 1. C5a (65-74)-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/C5a/Gα15 and CHO-K1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with a C5a receptor agonist, C5a (65-74). The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of C5a (65-74) (Mean ± SD, n = 2). The EC₅₀ of C5a (65-74) on C5a co-expressing with Gα15 in CHO-K1 cells was 16 nM. The S/B of C5a (65-74) on C5a co-expressing with Gα15 in CHO-K1 cells was 7.

Notes:

- EC₅₀ value is calculated with four parameter logistic equation:

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{((\text{LogEC}_{50} - X) * \text{HillSlope}))}$$
X is the logarithm of concentration. Y is the response
Y is RFU and starts at Bottom and goes to Top with a sigmoid shape.
- Signal to background Ratio (S/B) = Top/Bottom

IV. THAWING AND SUBCULTURING

Thawing Protocol

- Remove the vial from liquid nitrogen tank and thaw cells quickly in a 37°C water-bath.
- Just before the cells are completely thawed, decontaminate the outside of the vial with 70% ethanol and transfer the cells to a 15 ml centrifuge tube containing 9 ml of complete growth medium.
- Pellet cells by centrifugation at 200 x g force for 5 min, and remove the medium.
- Resuspend the cells in complete growth medium.
- Transfer the cell suspension to a 10 cm dish with 10 ml of complete growth medium.
- Grow the cells in incubator with 37°C, 5 %CO₂.
- Add antibiotic in the following day.

Sub-culturing Protocol

1. Remove the culture medium from cells.
2. Wash cells with PBS (pH=7.4) to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 ml of 0.05% (w/v) Trypsin- EDTA (GIBCO, Cat No. 25300) 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 force for 5min, and remove the medium.
6. Resuspend the cells in culture medium and add the cells suspension to new culture dish.
7. Grow the cells in incubator with 37°C, 5 %CO₂.

Subcultivation Ratio: 1:3 to 1:8 weekly.

Medium Renewal: Every 2 to 3 days

V. REFERENCES

1. Lee CH *et al.* (1995) Multiple regions of G alpha 16 contribute to the specificity of activation by the C5a receptor. *Mol Pharmacol.* 47(2):218-23.
2. Shum JK *et al.* (1995) The human chemoattractant complement C5a receptor inhibits cyclic AMP accumulation through Gi and Gz proteins. *Biochem Biophys Res Commun.* 208(1):223-9.
3. Manthey HD *et al.* (2009) Complement component 5a (C5a). *Int J Biochem Cell Biol.* 41(11):2114-7. Epub 2009 Apr 11.
4. Gueler F *et al.* (2008) Complement 5a receptor inhibition improves renal allograft survival. *J Am Soc Nephrol.* 19(12):2302-12. Epub 2008 Aug 27.

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