# Human Recombinant Motilin receptors MTLR Stable Cell Line

**Cat. No.: M00347** **Version 07012014**



## I. INTRODUCTION

Catalog Number: M00347
Cell Line Name: CHO-K1/Gα15/MTLR
Aliases: MTLR1; GPR38
GenBank Accession Number: NM_001507 (no expressed tags)
Host Cell line: CHO-K1/Gα15
Quantity: Two vials of frozen cells ($3\times10^6$ per vial)
Stability: Stable in culture for minimum of 20 passages
Application: Functional assay for MTLR receptor
Freeze Medium: 45% culture medium, 45% FBS, 10% DMSO
Propagation Medium: Ham's F12, 10% FBS, 400 μg/ml G418 and 100 μg/ml Hygromycin
Mycoplasma Status: Negative
Storage: Liquid nitrogen immediately upon receiving

## II. BACKGROUND

Motilin Receptors are Gq/11-protein-coupled receptors that mediate progastrokinetic effects. They are found at their highest concentrations in the nerves of the antral wall of the stomach and are also found at significant levels throughout the smooth muscle of the upper gastrointestinal (GI) tract and in the enteric nervous system. Motilin receptors promote gastric emptying after food intake and increase smooth muscle contraction in the GI tract.

*§: 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

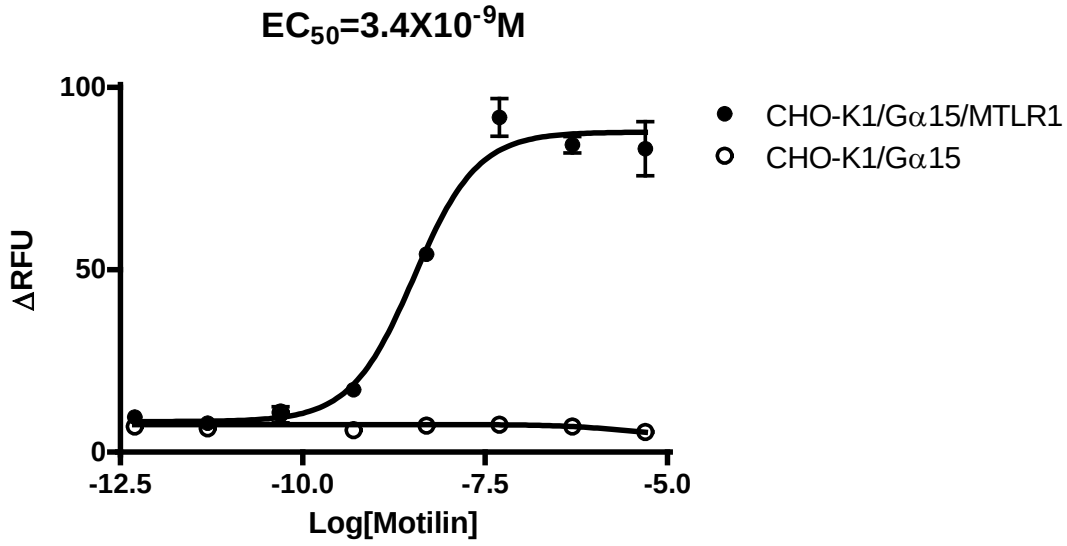


**Figure** Dose dependent stimulation of intracellular calcium mobilization upon treatment with its ligand Mltilin. The MTLR-expressing stable cell line (GenScript, Cat No: M00347) was loaded with Calcium-4 prior to stimulation with a MTLR receptor agonist, Motilin. The intracellular calcium mobilization was monitored by FLIPR. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of Motilin (Mean ± SD, n = 2).

**Notes:**

1. $EC_{50}$ value is calculated with four parameter logistic equation:
   Y=Bottom + (Top-Bottom)/(1+10^((Log$EC_{50}$-X)*HillSlope))
   X is the logarithm of concentration.
   Y is RFU and starts at Bottom and goes to Top with a sigmoid shape.
2. Signal to background Ratio (S/B) = Top/Bottom

## IV. THAWING AND SUBCULTURING

**Thawing Protocol**

1. Remove the vial from liquid nitrogen tank and thaw cells quickly in a 37°C water-bath.
2. 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.
3. Pellet cells by centrifugation at 200 x g force for 5 min, and remove the medium.
4. Resuspend the cells in complete growth medium.
5. Transfer the cell suspension to a 10 cm dish with 10 ml of complete growth medium.
6. Grow the cells in incubator with 37°C, 5 %$CO_2$.
7. In the following day, replace the cells with fresh medium contains antibiotic.

**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 %$CO_2$.

Subcultivation Ratio: 1:3 to 1:8 weekly.
Medium Renewal: Every 2 to 3 days

## V. REFERENCES

1. Feighner SD, Tan CP, McKee KK, Palyha OC, Hreniuk DL, Pong SS, Austin CP, Figueroa D, MacNeil D, Cascieri MA, Nargund R, Bakshi R, Abramovitz M, Stocco R, Kargman S, O'Neill G, Van der Ploeg LH, Evans J, Patchett AA, Smith RG, Howard AD. (1999) Receptor for motilin identified in the human gastrointestinal system. *Science*, 284: 2184-2188.
2. McKee KK, Tan CP, Palyha OC, Liu J, Feighner SD, Hreniuk DL, Smith RG, Howard AD, Van der Ploeg LH. (1997) Cloning and characterization of two human G protein-coupled receptor genes (GPR38 and GPR39) related to the growth hormone secretagogue and neurotensin receptors. *Genomics,* 46: 426-434.

**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

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