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**Human Recombinant Motilin receptors MTLR Stable Cell Line****Cat. No.: M00347****Version 07302020**

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**I. INTRODUCTION**

Catalog Number: M00347

Cell Line Name: CHO-K1/MTLR/G $\alpha$ 15

Aliases: MTLR1; GPR38

GenBank Accession Number: NM\_001507 (no expressed tags)

Host Cell line: CHO-K1/G $\alpha$ 15

Culture Properties: Adherent

Quantity: Two vials of frozen cells (>1 $\times$ 10<sup>6</sup> per vial)

Stability: Stable in culture for minimum of 16 passages

Application: Functional assay for MTLR receptor (Calcium flux assay)

Freeze Medium: 45% culture medium, 45% FBS (Cat. #10099-141, Gibco), 10% DMSO (Cat. #D2650, Sigma)

Propagation Medium: Ham's F-12K (Kaighn's) (Cat. #21127, Life Technologies), 10% FBS, 400  $\mu$ g/ml G418 (Cat. #10131-035, Gibco) and 100  $\mu$ g/ml Hygromycin B (Cat. #10687010, Invitrogen)

Mycoplasma Status: Negative\*

Storage: Liquid nitrogen immediately upon receipt

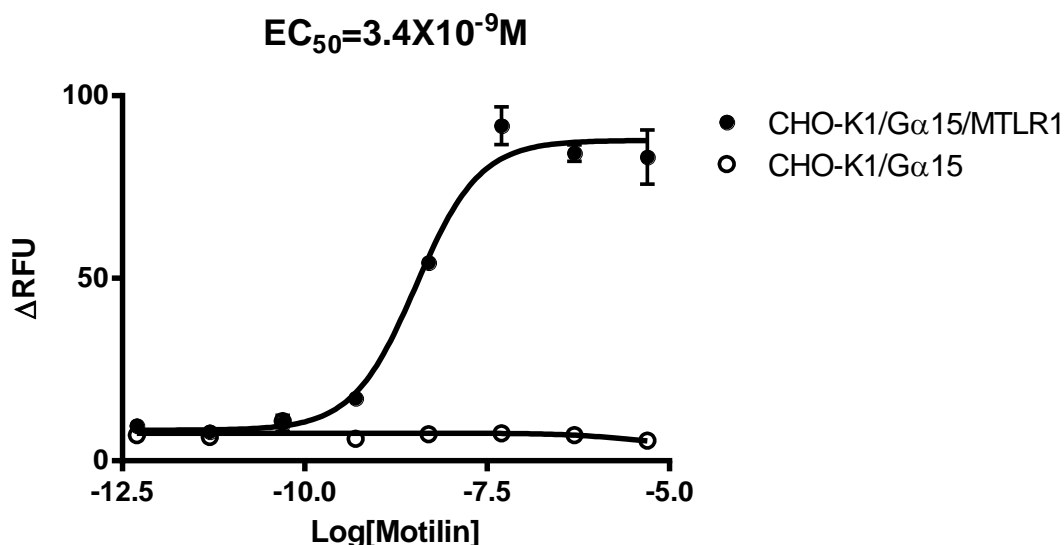
**II. BACKGROUND**

Motilin Receptors are Gq/11-protein-coupled receptors that mediate progastric 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.

\* The mycoplasma test was performed with MycoAlert™ PLUS Mycoplasma Detection Kit of Lonza.

### III. REPRESENTATIVE DATA

Calcium flux assay:



**Figure 1.** Dose dependent stimulation of intracellular calcium mobilization upon treatment with its ligand motilin. The MTLR-expressing stable cell line was loaded with Calcium-4 prior to being stimulated with agonist motilin. The intracellular calcium mobilization was monitored by FLIPR. The relative fluorescent units (RFU) were normalized and plotted against the log of the cumulative doses of motilin (Mean  $\pm$  SD, n = 2).

#### Notes:

1.  $EC_{50}$  value is calculated with four parameter logistic equation:  

$$Y = \text{Bottom} + \frac{(\text{Top} - \text{Bottom})}{(1 + 10^{-(\text{Log}EC_{50} - X) \cdot \text{HillSlope}})}$$
 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<sub>2</sub>.
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<sub>2</sub>.

Subcultivation Ratio: 1:3 to 1:8.

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.

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