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**Human Recombinant Chemokine Receptor-like 1 Stable Cell Line****Cat. No. M00301****Version 07292020**

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**I. INTRODUCTION**

Catalog Number: M00301

Cell Line Name: CHO-K1/CMKLR1/Gα15

Gene Synonyms: ChemR23, CMKLR1, DEZ, MGC126106, MGC126105, CHEMR23

Expressed Gene: Genbank Accession Number NM\_004072; no expressed tags

Host Cell: CHO-K1/Gα15

Culture Properties: Adherent

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

Stability: More than 16 passages

Application: Functional assay for CMKLR1 receptor (Calcium flux assay)

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

Complete Growth Medium: Ham's F-12K (Kaighn's) (Cat. #21127, Life Technologies), 10% FBS

Culture Medium: Ham's F-12K (Kaighn's), 10% FBS, 200 µg/ml Zeocin (Cat. #R250-01, Life Technologies), 100 µg/ml Hygromycin B (Cat. #10687010, Invitrogen)

Mycoplasma Status: Negative\*

Storage: Liquid nitrogen immediately upon receipt

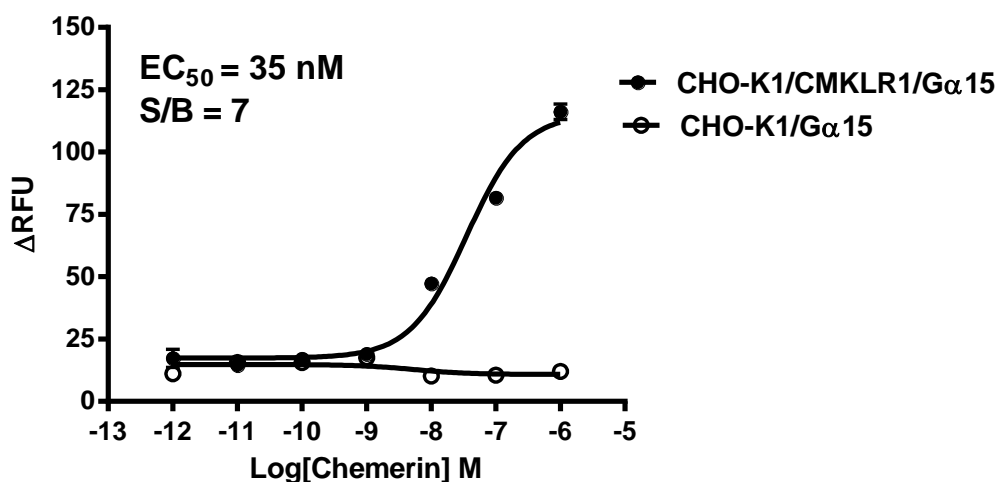
**II. BACKGROUND**

The orphan receptor ChemR23 (also referred to as CMKLR1 or DEZ) is a G-protein coupled receptor (GPCR) with homology to neuropeptide and chemoattractant receptors. ChemR23 is also a Gαi-linked receptor expressed primarily by monocytes, MΦs, and plasmacytoid dendritic cells. CMKLR1 has been shown to be expressed by DC generated *in vitro* from monocytes, to mediate their migration to the proteolytically regulated chemoattractant chemerin, and CMKLR1 may be a key mediator of pDC recruitment from the blood into tissue sites enriched in active chemerin.

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

### III. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by Chemerin in CHO-K1/CMKLR1/Gα15 cells



**Figure 1.** Chemerin-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/CMKLR1/Gα15 cells. The cells were loaded with Calcium-4 prior to being stimulated with agonist chemerin. The intracellular calcium change was measured by FLIPR<sup>TETRA</sup>. The relative fluorescent units (RFU) were normalized and plotted against the log of the cumulative doses of chemerin (Mean ± SD, n = 2). The EC<sub>50</sub> of chemerin on this cell was 35 nM.

#### Notes:

- EC<sub>50</sub> value is calculated with four parameter logistic equation:  

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{-(\text{LogEC}_{50} - X) \cdot \text{HillSlope}})$$

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 %CO<sub>2</sub>.
- Add antibiotic in the following day.

#### Sub-culturing Protocol

- Remove the culture medium from cells.
- 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

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