Human Recombinant Formylpeptide Receptor 1 Stable Cell Line
Cat. No. M00429 **Version 07302020**

I. INTRODUCTION

Catalog Number: M00429
Cell Line Name: CHO-K1/FPR1/Gα15
Gene Synonyms: FPR1; FMLP; FPR
Expressed Gene: Genbank Accession Number NM_002029; no expressed tags
Host Cell: CHO-K1/Gα15
Culture Properties: Adherent
Quantity: Two vials of frozen cells (>1×10^6 per vial)
Stability: More than 16 passages
Application: Functional assay for FPR1 receptor (Calcium flux assay, cAMP 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 formyl peptide receptors (FPR) are a class of G protein-coupled receptors involved in chemotaxis. FPR1 is a Gi-coupled GPCR. FPR1 is expressed in the vasculature, secretory epithelial cells, neurons and other tissues. FPR is involved in host defense against bacterial infection and in the clearance of damaged cells.

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

III. REPRESENTATIVE DATA

Calcium flux assay:

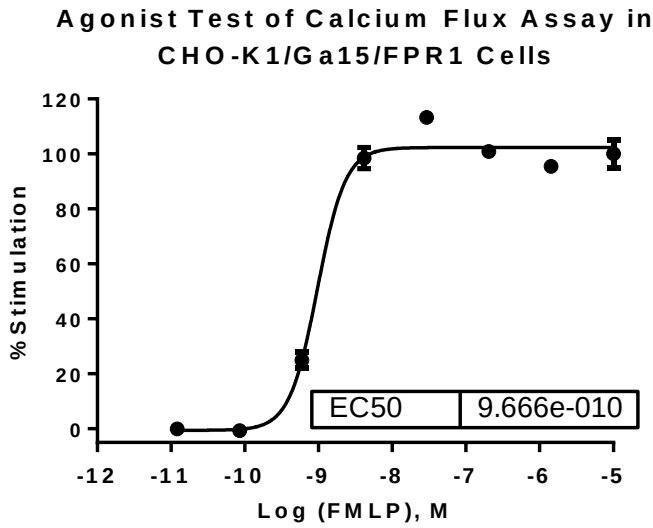

Figure 1. FMLP-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/FPR1/Gα15 cells. The cells were loaded with Calcium-4 prior to being stimulated with agonist FMLP. The intracellular calcium change was measured by FLIPR. The relative fluorescent units (RFU) were normalized and plotted against the log of the cumulative doses of FMLP (Mean ± SD, n = 2). The EC_{50} of FMLP on this cell was 0.97 nM.

Notes:

1. EC_{50} value is calculated with four parameter logistic equation:
   Y=Bottom + (Top-Bottom)/ (1+10^ ((LogEC_{50}-X)*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.

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. 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 %CO_2.

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

V. REFERENCES

1. Torres, M. and R. D. Ye. (1996) Activation of the mitogen-activated protein kinase pathway by fMet-leu-Phe in the absence of Lyn and tyrosine phosphorylation of SHC in transfected cells. *J Biol Chem.* 271(22): 13244-9.
2. Migeotte I, Communi D, Parmentier M. (2006) Formyl peptide receptors: a promiscuous subfamily of G protein-coupled receptors controlling immune responses. *Cytokine Growth Factor Rev.* 17(6): 501–19.
3. Ye RD, Boulay F, Wang JM, *et al.* (2009) International Union of Basic and Clinical Pharmacology. LXXIII. Nomenclature for the formyl peptide receptor (FPR) family. *Pharmacol. Rev*. 61(2): 119–61.

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