# Human Recombinant Free Fatty Acid Receptor 1 Stable Cell Line

**Technical Manual No. TM0420** **Version 06042010**



## I. Introduction

Catalog Number: M00273

Cell Line Name: CHO-K1/FFA1/Gα15

Expressed Gene: GenBank Accession Number NM_005303; no expressed tags

Host Cell: CHO-K1

Quantity: 2 vial ($3\times10^6$ per vial) frozen cells

Stability: 16 passages

Applications: Functional assays for FFA1 receptor (GPR40)

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

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

Culture Medium: Ham's F12, 10% FBS, 200 μg/ml Zeocin, 100 μg/ml Hygromycin B

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

## II. Background

Free fatty acid G protein coupled receptor family consists of four members and plays significant roles in nutrition regulation. GPR40 (FFA1) and GPR120 are activated by medium and long-chain FFAs, whereas GPR41 and GPR43 (FFA2) can be activated by short-chain FFAs. FFA1 is preferentially expressed in pancreatic beta-cells and mediates the majority of the effects of FFAs on insulin secretion. Researches show that FFA1 is a potential therapeutic target and plays an important role in the chain of events linking obesity and type2 diabetes.



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## III. Representative Data

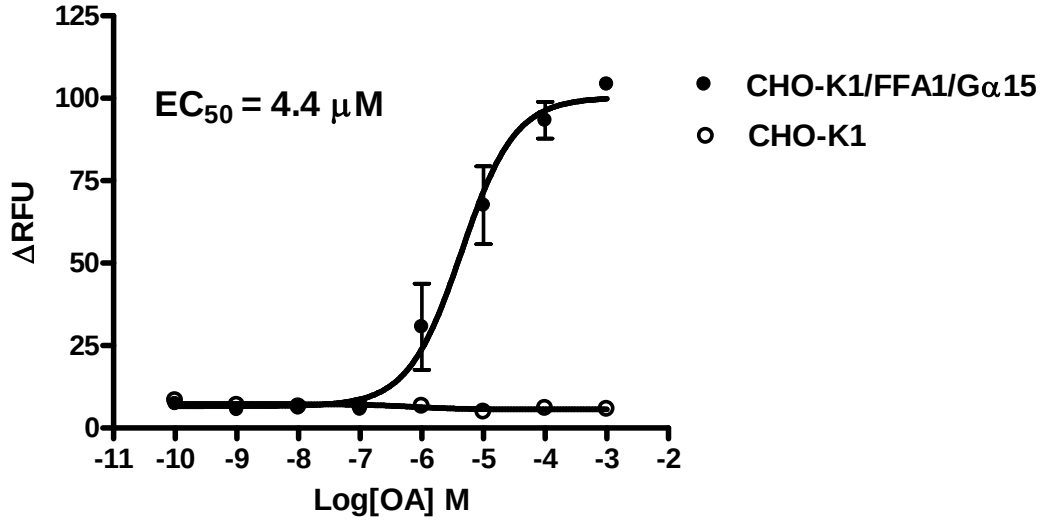


**Figure:** Shown above are the intracellular calcium responses of CHO-K1 cells stably expressing Homo sapiens free fatty acid receptor 1 (FFA1/GPR40) and of untransfected control cells. Cells were loaded with Calcium-4 and then stimulated with the indicated concentrations of Oleic acid. Calcium responses were recorded on a FlexStation plate reader. Data represent the average +/- standard deviation of triplicate determinations.

## 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 discard the medium.
4. Resuspend the cells in complete growth medium.
5. Add 2 ml of the cell suspension per well in a 6 well-plate.
6. Add Hygromycin B and Zeocin to concentrations of 100 µg/ml and 200 µg/ml respectively the following day.

Subculturing: Protocol

1. Remove and discard culture medium.
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 to 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 clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting, centrifuge the cells 200 x g force for 5min, and discard the medium.
5. Resuspend the cells in complete growth medium with Hygromycin B and Zeocin and add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.



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

## V. References

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3. Thompson, A. L., Lim-Fraser, M. Y. C., Kraegen, E. W., and Cooney, G. *J. (2000) Am. J. Physiol. 279, E577–E584*

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