# Human Recombinant GPR40 Stable Cell Line

**Technical Manual No. TM0287** **Version 05152009**



## I. Introduction

G-protein coupled receptor 40 (GPR40), also named "Free Fatty Acid Receptor 1" (FFA Receptor 1), is specifically expressed in the brain and pancreas. In the pancreas, abundant GPR40 is localized to insulin-producing beta cells. Free fatty acids regulate insulin secretion from pancreatic β cells through GPR40, indicating that GPR40 agonists and/or antagonists have potential for the development of new anti-diabetic drugs. GPR40 is a G protein-coupled receptor that signals through Gq, which makes it suitable for β-lactamase technology and calcium influx assay technology. Some literatures showed that GPR40 overexpression in breast cancer cells amplified oleate-induced proliferation, whereas silencing the GPR40 gene using RNAi decreased it. These results suggest that GPR40 is implicated in the control of breast cancer cell growth by fatty acids and that GPR40 may provide a link between fat and cancer. This manual describes establishment of a cell line and a protocol of pharmacologically validated human G-protein coupled receptor 40 (Genebank Accession Number: NM_005303).

## II. Cell Line Information

- Catalog Number: M00137
- Cell Line Name: HEK/GPR40/NFAT/β-Lac
- Description:
  Human GPR40 is amplified by PCR using a high fidelity enzyme and subcloned into the pcDNA3.1/hygro(+) mammalian expression vector. The full-length ORF has been confirmed by sequencing. The GPR40 cell line is created by transfection of pcDNA3.1/GPR40 in a parental cell line, HEK293/NFAT/β-lactamase. The transfected cells are stably selected by 100 μg/ml hygromycin. Single cell clones with high GPR40 receptor inducibility and low β-lactamase background are isolated using ring cloning. The clones with largest dynamic ranges in β-lactamase are chosen for pharmacological and stability studies.
- Function: Cell based, functional assay for GPR40
- Quantity: 2 vial ($2 \times 10^6$/vial) frozen cells
- Host Cell: HEK293
- Cell Phenotype: Adherent/epithelial
- Mycoplasma: Negative
- Recommended Storage: Liquid nitrogen, upon delivery
- Propagation Medium: DMEM, 10% FBS, 100 μg/ml hygromycin.



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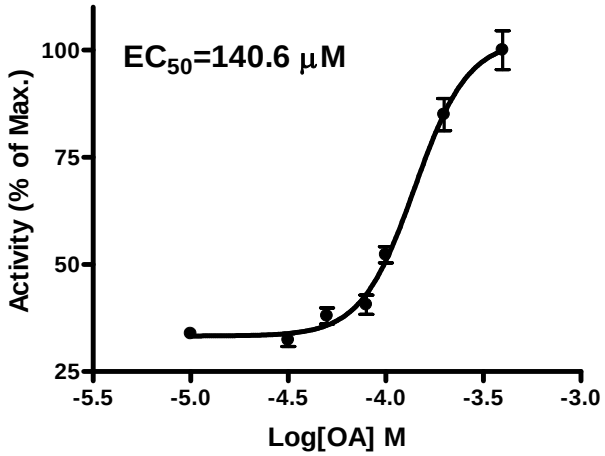
## III. Oleic Acid Dose Response of HEK293/GPR40/NFAT/β-Lac Stable Cell Line and Assay Procedure




1. Seed 25,000 cells per well in Growth Medium (100 µl per well) into 96-well tissue culture treated black-wall, clear-bottom plates (Costar #3603) after trypsinization. Prepare some wells with medium alone (no cells) to use for determining plate background.
2. Culture cells in 5% $CO_2$ at 37°C. Allow cells to reach ≈90% confluence.
3. 12-24 hours before the assay, replace Growth Medium with 100 µl/well serum-free DMEM. Be careful not to disturb the cells.
4. Prepare ligand solution in serum-free DMEM (10X).
5. Add 10 µl of 10X ligand solution to wells for stimulation and 10 µl of serum-free DMEM per well for non-stimulated control.
6. Incubate cells in 5% $CO_2$ at 37°C for 5-6 hours.
7. Load cells with 2 µM CCF4/AM as described in CCF4 Loading Protocol.
8. Incubate the plate at room temperature for 60-120 minutes without shaking.
9. Read with analyst HT plate reader or observe under fluorescence microscope for 60-200 minutes after CCF-4 loading.

## IV. References

1. Briscoe, C. P., M. Tadayyon, *et al*. (2003). The orphan G protein-coupled receptor GPR40 is activated by medium and long chain fatty acids. *J. Biol. Chem.* 278(13): 11303-11.
2. Yasuaki Itoh, Yuji Kawamata, *et al* (2003). Free fatty acids regulate insulin secretion from pancreatic b cells through GPR40. *Nature* 422(13): 173-176
3. Steneberg, P., N. Rubins, *et al*. (2005). The FFA receptor GPR40 links hyperinsulinemia, hepatic steatosis, and impaired glucose homeostasis in mouse. *Cell Metab.* 1(4): 245-58.
4. Hardy, S., G. G. St-Onge, *et al*. (2005). Oleate promotes the proliferation of breast cancer cells via the G protein-coupled receptor GPR40. *J. Biol. Chem.* 280(14): 13285-91.



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# V. Appendix

## Cell Culture Conditions

**Complete Culture Medium:**
DMEM: 90%, FBS: 10%, L-glutamine: 2.0 mM, Amp: 100 μg/ml, Strep: 100 μg/ml, hygromycin: 100 μg/ml

**Serum-free DMEM:**

Same as above but with no FBS, 0.1% BSA

**Freezing Medium:**

Complete culture medium plus 20% FBS and 10% DMSO

**Thawing Cells:**

1. Quickly thaw frozen cells in a 37°C water bath, agitating continuously.
2. Using a 1 ml pipette, slowly pipet the cells up and down five times and add, drop by drop, to a 15 ml centrifuge tube containing 5 ml of fresh prewarmed complete DMEM medium. Then centrifuge at 1,000 rpm for five minutes.
3. Discard the supernatant medium and resuspend the cell pellet in 5 ml of fresh prewarmed complete DMEM medium. Transfer cells to a T25 flask and incubate at 37°C with 5% $CO_2$ until the cells reach >90% confluence. The recovery rate for frozen cells is usually 90% or above.

**Subculturing:**

When the cells reach confluence, they need split. This cell line is normally split twice weekly at 1:8 to 1:15 dilutions.

1. Carefully aspirate all the media. Gently rinse the cell layer with appropriate amount of 0.2% trypsin-EDTA, and aspirate it off.
2. Wait for about 1-3 minutes. Dislodge the cells by gently tapping the sides of flask or dish.
3. Resuspend cells with appropriate amount of complete DMEM medium, and split cells as desired.

**Changing Medium:**
This is normally done every other day.

1. Gently aspirate off medium.
2. Transfer fresh warm complete DMEM medium (37°C) into a flask (5 ml for T25 and 10 ml for T75).

**Freezing Cells:**

1. Repeat steps 1-3 of subculturing section.
2. Centrifuge down the cells at 1,000 rpm for five minutes.
3. Aspirate off the supernatant and resuspend the cells in fresh freezing medium at a density of 2-3 × $10^6$ cells/ml. Add 1 ml cells per cryogenic vial.
4. Put the cryogenic vial of cells into cryo freezing container. Then transfer the container to a -80°C environment and leave it there overnight.
5. Transfer cryogenic vial into liquid nitrogen (-196°C).

## Reagents & Consumables:

1. DMEM: Dulbecco's Modified Eagle Medium powder, high glucose (Gibco BRL, Cat #12100-046)
2. FBS: Fetal Bovine Serum (Hyclone, Cat #CH30160.03)
3. L-Glutamine: 200 mM (Gibco BRL, Cat # 25030-081)
4. Ampicillin: 50 mg/ml (Sigma A-9518)



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5. Streptomycin Sulfate: 50 mg/ml (Gibco BRL, Cat # 11860-038)
6. Hygromycin B in PBS, 50 mg/ml (Invitrogen, Cat #10687-010)
7. Trypsin: 1:250 rom Bovine Pancreas (Gibco BRL, Cat # 27250016)
8. DMSO: dimethyl sulphoxide (Sigma, Cat #D8418)
9. Hepes: Sigma Cat #H-3375
10. CCF4: (Invitrogen, Cat #K1096)
11. Venor$^{@}$GeM Mycoplasma Detection kit: Minerva Biolabs Cat #11-1050
12. 96 Well Plate: Costar, Cat# 3603, Blackwall/clear bottom, Polystyrene, sterilized.

**Media and Solutions:**

1. PBS (for preparation of 500 ml)

    1) KCl: 0.1 g
    2) $KH_2PO_4$: 0.1 g
    3) NaCl: 4.0 g
    4) $Na_2HPO_4.12H_2O$: 1.4425 g

    Dissolve the above components in double-distilled water ($ddH_2O$) and adjust pH to 7.4 with 0.1 N NaOH. Add $ddH_2O$ to the final volume of 500 ml. Autoclave and store at 4°C.

2. Trypsin-EDTA (for preparation of 100 ml)

    1) Trypsin: 0.25 g
    2) 2%EDTA: 2 ml
    3) PBS: 98 ml

    Dissolve trypsin in 2%EDTA and PBS completely; sterilize the solution by passing through a 0.20 μm membrane filter; store at 4°C.

3. Culture medium (for preparation of 1 L)

    1) Measure out 950 ml distilled water to dissolve the media components, stirring gently until the solution becomes clear.
    2) Add $NaHCO_3$ 3.7 g for high glucose DMEM
    3) Adjust pH of medium to 0.2-0.3 below the desired final working pH (using 1 N NaOH or 1 N HCL is recommended). Add slowly while stirring.
    4) Dilute to 1 liter with $ddH_2O$.
    5) Sterilize the medium immediately using the method of membrane filtration. Store at 4°C

4. Ampicillin/Streptomycin 50 mg/ml

Dissolve 1 g ampicillin or streptomycin in 20 ml $ddH_2O$ and sterilize the solution by membrane filtration using 0.20 μm filter. Aliquot and store at 4°C for short-term conservation and -20°C for long term conservation.

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