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ChemiScreen™ CALCIUM-OPTIMIZED STABLE CELL LINE HUMAN RECOMBINANT GPR40 FREE FATTY ACID RECEPTOR

CATALOG NUMBER:	HTS038C	**QUANTITY:**	2 vials, 1 mL per vial
LOT NUMBER:		**CONCENTRATION:**	2 x 10^6 cells/mL

BACKGROUND:

GPR40 is a G_i and G_q-coupled GPCR that is activated by free long-chain fatty acids of 8 or more carbons. Pancreatic islets express GPR40, and GPR40 appears to regulate insulin release from islets in response to long-chain fatty acids in the plasma (reviewed in Brown *et al*., 2005). Chemicon's cloned human GPR40-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant GPR40 expression on the cell surface and contains high levels of the promiscuous G protein G□15 to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for antagonists of interactions between GPR40 and its ligands.

APPLICATIONS:

Calcium flux assay, ligand binding assays

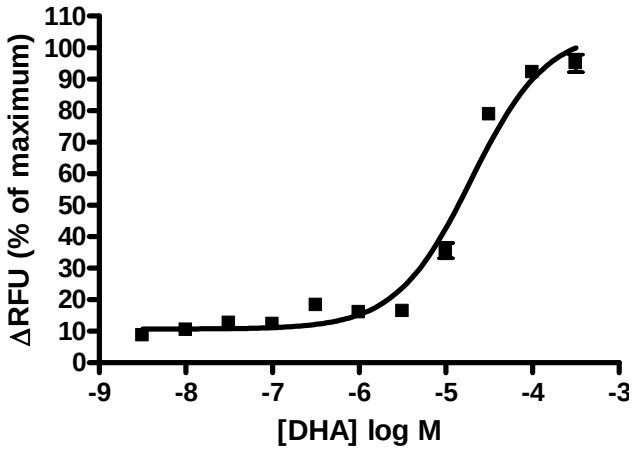

Figure 1. Calcium flux in GPR40–expressing Chem-1 cell line induced by Docosahexaenoic acid (DHA). GPR40–expressing Chem-1 cells were loaded with Fluo-4 and calcium flux in response to DHA ($10^{-3.5}$ to $10^{-8.5}$ M) was determined on a Molecular Devices FLIPR$^{TETRA_{TM}}$.

SPECIFICATIONS: EC50 for calcium mobilization by Docosahexaenoic acid: ~ 22 µM

HOST CELLS: Chem-1, an adherent cell line expressing the promiscuous G-protein, Gα15.

TRANSFECTION: Full-length human GPR40 cDNA (Accession Number: NM_005303)

PRESENTATION:

Cells are frozen at 2 x 10^6 cells/mL in DMEM/20% fetal bovine serum/100 U/ml penicillin and streptomycin/10% DMSO. Cell line tests negative for mycoplasma.

STORAGE/HANDLING:

1. Immediately upon receipt, thaw cells or place cells in liquid nitrogen. Maintain frozen in liquid nitrogen for up to 5 years.
2. Thaw cells rapidly by removing from liquid nitrogen and immediately immersing in a 37ºC water bath. Immediately after ice has thawed, sterilize the exterior of the vial with 70% ethanol. Transfer contents of the vial to a T75 flask containing growth

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media. Place the flask in a humidified incubator at 37ºC with 5% CO_2.

3. After 8-24 h, all live cells will be attached. Viability of the cells is expected to be 50-80%. At this time, replace media to remove residual DMSO, and return to incubator.
4. When cells are approximately 80% confluent, passage the cells as follows: Remove media and wash once with HBSS without Ca^{++} and Mg^{++} (10 mL/T75). Add 0.05% trypsin/0.2 g/L EDTA (1 mL/T75) and place in humidified incubator at 37ºC with 5% CO_2 until cells begin to round up and detach (5-10 minutes). Gently rap the side of the flask to dislodge the cells. Neutralize trypsin by addition of 4 mL Chem-1 Growth Media per 1 mL trypsin.
5. Cells are typically passaged 1:10 every 3-4 days. Passaging ratio may be varied according to requirements of the investigator.
6. Frozen stocks of cells should be prepared at the earliest passage possible after thawing, as follows: Count detached cells (prepared as in Step 4). Centrifuge cells at 200 x g for 5 min. Resuspend cells at 5 x 10^6 cells/mL in Chem-1 Freezing Media (cell densities of 2-10 x 10^6 are also acceptable if necessary). Dispense 1 mL aliquots into cryopreservation vials. Freeze the cells by a controlled rate process, such as in an isopropanol-jacketed container placed at –70°C overnight. Store the vials in liquid nitrogen.
7. Use of cells immediately after thawing is feasible for some cell lines and is being further validated. Some cell lines may need to be passaged at least once after thawing prior to use in calcium flux assays. Cells should be resuspended in Chem-1 Plating Media for plating for calcium assay.

MEDIA:

Chem-1 Growth Media*:*
DMEM with 4.5 g/L glucose and 4 mM glutamine (Millipore SLM-020-A)
10% heat-inactivated FBS
1x Nonessential amino acids (from 100x stock, Millipore TMS-001-C)
10mM HEPES (from 1 M HEPES, Millipore TMS-003-C)
1x Pen-Strep (from 100x stock, Millipore TMS-AB2-C)
250 g/mL Genetecin/G-418

Chem-1 Plating Media:
DMEM with 4.5 g/L glucose and 4 mM glutamine
10% heat-inactivated FBS
1x NEAA
10mM HEPES
1x Pen-Strep

Chem-1 Freezing Media:
DMEM with 4.5 g/L glucose and 4 mM glutamine
20% heat-inactivated FBS
1x NEAA
10mM HEPES
1x Pen-Strep
10% DMSO (cell culture grade)

REFERENCES:

Brown A.J. et al. (2005) A family of fatty acid binding receptors. *DNA Cell Biol.* 24: 54-61.

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