

PRODUCT DATASHEET

Ready-to-Assay™ FFA1/GPR40 Free Fatty Acid Receptor Frozen Cells**CATALOG NUMBER: HTS038RTA****Lot: 038020516****CONTENTS:** Pack contains 2 vials of mycoplasma-free cells, 1 ml per vial. Fifty (50) mL of Media Component.**STORAGE:** Vials are to be stored in liquid N₂. Media Component at 4°C (-20°C for prolonged storage).**BACKGROUND**

Ready-to-Assay™ GPCR frozen cells are designed for simple, rapid calcium assays with no requirement for intensive cell culturing. The freezing conditions have been optimized to provide cells with high viability and functionality post-thaw. The user simply thaws the cells and resuspends them in media, dispenses cell suspension into assay plates and, following overnight recovery, assays for calcium response.

GPR40 is a Gi and Gq-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). The 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 Gα15 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.

USE RESTRICTIONS

Please see User Agreement (Label License) for further details. ***One such restriction is that the contents of the supplied vial(s) are limited to a single use and shall not be propagated and/or re-frozen by licensee.***

WARNINGS

For Research Use Only; Not for Use in Diagnostic Procedures
Not for Animal or Human Consumption

GMO

This product contains genetically modified organisms.
Este producto contiene organismos genéticamente modificados.
Questo prodotto contiene degli organismi geneticamente modificati.
Dieses Produkt enthält genetisch modifizierte Organismen.
Ce produit contient organismes génétiquement des modifiés.
Dit product bevat genetisch gewijzigde organismen.
Tämä tuote sisältää geneettisesti muutettuja organismeja.
Denna produkt innehåller genetiskt ändrade organismer.

APPLICATIONS

Calcium Flux Assays: Luminescent Mode and Fluorescent Mode; cAMP accumulation

APPLICATION DATA

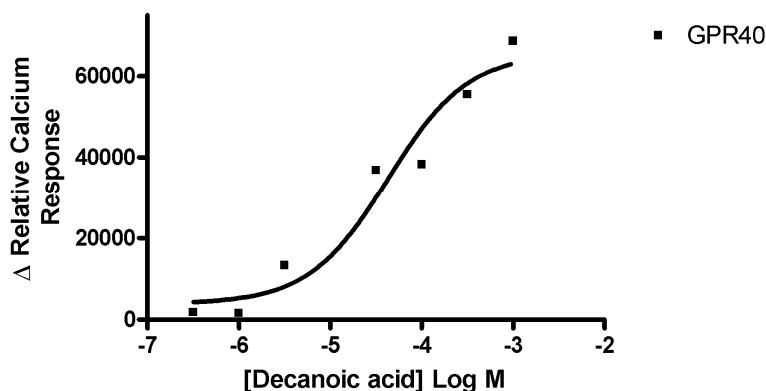


Figure 1. Representative data for activation of GPR40 receptor. Calcium flux in GPR40-expressing Chem-1 cell line induced by decanoic acid. GPR40-expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPRETETRA.

Table 1. Comparison of EC₅₀ values of FFA1-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Decanoic Acid	Calcium Flux - Fluorescence	40	Eurofins Internal Data

ASSAY SETUP

Fluorescence

Table 3. Settings for FLIPR^{TETRA}® with ICCD camera option

Option	Setting
Read Mode	Fluorescence
Ex/Em	Ex470_495 / Em515_575
Camera Gain	2000
Gate Open	6 %
Exposure Time	0.53
Read Interval	1s
Dispense Volume	50 µl (25 µl for 384-well)
Dispense Height	95 µl (50 µl for 384-well)
Dispense Speed	50 µl/sec
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

Table 4. Assay Materials (Not provided)

Description	Supplier and Product Number
HBSS	Invitrogen: 14025
HEPES 1M Stock	EMD Millipore: TMS-003-C
Probenicid	Sigma: P8761

Quest Fluo-8™, AM

AAT Bioquest: 21080

GW9508 ligand

Tocris: 2649

Non-Binding 96/384 well Plates (for ligand prep)

Corning: 3605/ 3574

Black (clear Bottom) cell assay plates

Corning: 3904/ 3712

Coelenterazine-h (250µg). Prepare to 10mM

Promega: S2011

HOST CELL

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

EXOGENOUS GENE EXPRESSION

Human GPR40 cDNA (Accession Number: NM_005303; see CODING SEQUENCE below) and promiscuous G protein are expressed in a bicistronic vector

CODING SEQUENCE

```

ATG GAC CTG CCC CCG CAG CTC TCC TTC GGC CTC TAT GTG GCC GCC TTT GCG CTG GGC TTC CCG CTC
M D L P P Q L S F G L Y V A A F A L G F P L
AAC GTC CTG GCC ATC CGA GGC GCG ACG GCC CAC GCC CGG CTC CGT CTC ACC CCT AGC CTG GTC TAC
N V L A I R G A T A H A R L R L T P S L V Y
GCC CTG AAC CTG GGC TGC TCC GAC CTG CTG CTG ACA GTC TCT CTG CCC CTG AAG GCG GTG GAG GCG
A L N L G C S D L L L T V S L P L K A V E A
CTA GCC TCC GGG GCC TGG CCT CTG CCG GCC TCG CTG TGC CCC GTC TTC GCG GTG GCC CAC TTC TTC
L A S G A W P L P A S L C P V F A V A H F F
CCA CTC TAT GCC GGC GGG GGC TTC CTG GCC GCC CTG AGT GCA GGC CGC TAC CTG GGA GCA GCC TTC
P L Y A G G G F L A A L S A G R Y L G A A F
CCC TTG GGC TAC CAA GCC TTC CGG AGG CCG TGC TAT TCC TGG GGG GTG TGC GCG GCC ATC TGG GCC
P L G Y Q A F R R P C Y S W G V C A A I W A
CTC GTC CTG TGT CAC CTG GGT CTG GTC TTT GGG TTG GAG GCT CCA GGA GGC TGG CTG GAC CAC AGC
L V L C H L G L V F G L E A P G G W L D H S
AAC ACC TCC CTG GGC ATC AAC ACA CCG GTC AAC GGC TCT CCG GTC TGC CTG GAG GCC TGG GAC CCG
N T S L G I N T P V N G S P V C L E A W D P
GCC TCT GCC GGC CCG GCC CGC TTC AGC CTC TCT CTC CTG CTC TTT TTT CTG CCC TTG GCC ATC ACA
A S A G P A R F S L S L L L F F L P L A I T
GCC TTC TGC TAC GTG GGC TGC CTC CGG GCA CTG GCC CGC TCC GGC CTG ACG CAC AGG CGG AAG CTG
A F C Y V G C L R A L A R S G L T H R R K L
CGG GCC GCC TGG GTG GCC GGC GGG GCC CTC CTC ACG CTG CTG CTC TGC GTA GGA CCC TAC AAC GCC
R A A W V A G G A L L T L L L C V G P Y N A
TCC AAC GTG GCC AGC TTC CTG TAC CCC AAT CTA GGA GGC TCC TGG CGG AAG CTG GGG CTC ATC ACG
S N V A S F L Y P N L G G S W R K L G L I T
GGT GCC TGG AGT GTG GTG CTT AAT CCG CTG GTG ACC GGT TAC TTG GGA AGG GGT CCT GGC CTG AAG
G A W S V V L N P L V T G Y L G R G P G L K
ACA GTG TGT GCG GCA AGA ACG CAA GGG GGC AAG TCC CAG AAG TAA
T V C A A R T Q G G K S Q K .

```

RELATED PRODUCTS

PRODUCT NUMBER

HTSCHEM-1

HTS038M

DESCRIPTION

ChemiScreen™ Chem-1 Parental Cell Line (control cells)

ChemiScreen™ GPR40 Free Fatty Acid Receptor Membrane Prep

REFERENCES

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

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION

Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

No part of these works may be reproduced in any form without permission in writing.

User Agreement (Label License)

In addition to the General Terms and Conditions section, these specific terms also apply for **Ready-to-Assay™ ChemiBrite™ FFA1/GPR40 Free Fatty Acid Receptor Frozen Cells, Product No. HTS038LRTA**

BY USING THIS PRODUCT LICENSED TO YOU (“LICENSEE”) HEREUNDER, YOU ARE HEREBY REPRESENTING THAT YOU HAVE THE RIGHT AND AUTHORITY TO LEGALLY BIND YOURSELF OR YOUR COMPANY, AS APPLICABLE, AND ARE CONSENTING TO BE LEGALLY BOUND BY ALL OF THE TERMS OF THIS USER AGREEMENT (“AGREEMENT”). IF YOU DO NOT AGREE TO ALL THESE TERMS, DO NOT USE THE PRODUCT, AND IMMEDIATELY RETURN SUCH PRODUCTS TO THE APPLICABLE SELLER FOR A REFUND. This is a legal agreement between Licensee and Eurofins Pharma Bioanalytics Services US Inc. governing use of the Ready-to-Assay Cells products and/or any accompanying operating/use protocols (the “Product(s)”) provided to Licensee.

LICENSEE shall obtain no ownership interest in the Product or use/culture protocols accompanying the Product other than through the perpetual limited license granted herein. If the Product is licensed through an authorized Eurofins Pharma Bioanalytics Services US Inc. distributor, Licensee shall be obligated to disclose its identity to Eurofins Pharma Bioanalytics Services US Inc. to insure compliance with this User Agreement.

Limited License and Restrictions. Pursuant to the terms and conditions of this Agreement, Eurofins Pharma Bioanalytics Services US Inc. conveys to Licensee the non-exclusive and non-transferable right to use the Licensed Product only for Research Purposes conducted by Licensee (whether Licensee is an academic user or a for-profit entity). “Research Purposes” means any biological research and development application or use, including without limitation, developing, demonstrating or validating biological assays, life sciences and/or pharmaceutical research. “Research Purposes” excludes applications outside biology (including but not limited to consumer electronics or materials sciences), and specifically excludes the following applications of whatever kind or nature: Clinical Diagnostics (any use of a product or service for clinical diagnosis where data from an individual’s sample is given to such individual or used for the purpose of diagnosis or treatment of a medical condition in such individual, where that result may be used in the treatment of such individual), therapeutics, clinical imaging, environmental testing and cosmetics. Contents of the supplied vial(s) are limited to a single use and shall not be propagated and/or re-frozen by licensee. Licensee cannot sell or otherwise transfer (a) this Product or (b) materials made using this Product to a third party. Licensee may transfer information or materials made through use of this Product to a scientific collaborator, provided that such transfer is not for the commercial purposes, and that such collaborator agrees in writing: (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for Research Purposes and not for commercial purposes. Commercial purposes means any activity by a user of the Product for consideration that may include, but is not limited to: (1) operating a service business that uses the Products to develop information or data which is resold for research and development applications; (2) use of the Product in manufacturing; (3) use of the Product for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the Product, whether or not such Product is resold for use in research. Licensee expressly represents and warrants to Eurofins Pharma Bioanalytics Services US Inc. that Licensee will properly test and use any Product purchased from Eurofins Pharma Bioanalytics Services US Inc. or its affiliated companies in accordance with the practices of a reasonable person who is an expert in the field and in strict compliance with all applicable laws and regulations, now and hereinafter enacted. Licensee agrees to comply with instructions, if any, furnished by Eurofins Pharma Bioanalytics Services US Inc. relating to the use of the Product and to not misuse the Product in any manner. Licensee shall not reverse engineer, disassemble or modify the Product or create any derivative works of the written materials accompanying the Product, including but not limited to any material data sheets or similar materials with respect to the Products’ specifications. Licensee acknowledges that

Eurofins Pharma Bioanalytics Services US Inc. or its affiliated companies retains ownership of all patents, copyrights, trademarks, trade secrets and other proprietary rights relating to or residing in the Product or any portion thereof.

Term and Termination. This Agreement commences upon Licensee's use of the Products, and shall remain in effect in perpetuity unless terminated sooner as set forth hereunder. Eurofins Pharma Bioanalytics Services US Inc. may terminate this Agreement immediately if Licensee breaches any provision herein. Upon any such termination, all rights granted to Licensee hereunder will immediately terminate, and Licensee shall immediately cease using the Product and, at Eurofins Pharma Bioanalytics Services US Inc.'s option, return or destroy all Products (certifying such destruction to Eurofins Pharma Bioanalytics Services US Inc. in writing).

Assignment. Licensee shall not sublicense, assign (by operation of law or otherwise) or otherwise transfer this Agreement or any of the rights or licenses granted under this Agreement without the prior written consent of Eurofins Pharma Bioanalytics Services US Inc.. Any attempted assignment, sublicense or transfer by Licensee without such consent shall be null and void.

Eurofins Pharma Bioanalytics Services US Inc. is an independent member of Eurofins Discovery Services