TECHNICAL DATA SHEET

AequoZen®

Caution: For Laboratory Use. A research reagent for research purposes only

Human Free Fatty Acid FFA1 (GPR40) Receptor, γ-Irradiated Frozen Cells

Product No.: ES-652-AF

Lot No.: 533-483-A

Material Provided

Cells:	1 x 1 mL frozen aliquot
Format:	~10 x 10^6 cells/mL in Ham's F12, 10% FBS with 10 % DMSO.

Product Information

Cellular Background:	CHO-K1
Parental Frozen Cells (control):	A12 (Cat # ES-000-A12F)
Frozen Cells Info:	Frozen recombinant, γ–irradiated CHO-K1 cells expressing mitochondrially-targeted Aequorin and the human Free Fatty Acid FFA1 (GPR40) receptor.
DNA Sequence:	Identical to coding sequence of GenBank BC120944.1.
Corresponding Protein Sequence:	Identical to GenBank AAI20945.1.
Storage Conditions:	Store in liquid nitrogen (vapor phase) immediately upon receipt, or maximum 15 days at -80°C. AequoZen® is designed for single use only. Do not refreeze.

Quality Control

EC_{50} for a reference agonist is determined using an AequoScreen® assay (Figure 1). Mycoplasma test is performed using MycoAlert® Mycoplasma detection kit. We certify that these results meet our quality release criteria.

Docosahexanoic Acid (EC_{50}):	23 µM
Mycoplasma:	This cell line tested negative for Mycoplasma.

PerkinElmer®
For the Better

Recommended Thawing Conditions and Handling of Frozen Cells

- Carefully follow instructions below to obtain the expected results. Most Frozen cells are intended to be assayed immediately upon thawing. Exceptionally, where specified, some frozen cell products require an overnight incubation in Cell Medium to enable them to perform optimally.

- The recommended media catalogue number and supplier reference information are listed in this Product Technical Data Sheet (last page). Media composition is specifically defined for each cell type and receptor. The use of incorrect media or component substitutions can lead to altered product performance. Additionally, the instructions for the preparation of ligands must be carefully followed to avoid ligand precipitation, degradation or adsorption. Inappropriate preparation may result in a non-representative pharmacology.

- The complete thawing procedure must not exceed 30 min. Cell viability below 90% upon thawing may indicate that the Frozen cells were affected by incorrect thawing procedure and may yield to lower performance. Ensure the cells are not clumped and are evenly distributed in the assay plates. **Gently** pipet up and down if cells are clumped before dispensing the cells. Frozen cells **cannot** be re-frozen.

Assay Medium (for immediate thaw and use): AequoScreen® Assay Buffer (see below)

Cell Medium (for overnight incubation prior to use): Ham's F-12, 10% FBS

Thawing Cells:

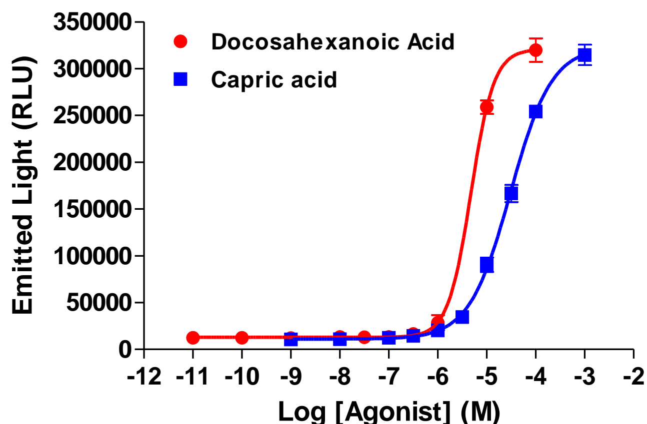
- Using appropriate personal protective equipment, rapidly place the frozen aliquot in a 37°C water bath (do not submerge) until its content is thawed completely. Immediately remove from water bath, spray aliquot with 70% ethanol and wipe excess. Under aseptic conditions using a sterile pipette, **gently** resuspend the cells in the cryovials and transfer content to a sterile centrifuge tube containing 10 mL of the Assay or Cell Medium **pre-warmed to 37°C**, and centrifuge (150 x g, 5 min.). Do not exceed the recommended centrifugal force. Discard supernatant using a sterile pipette. Gently resuspend cell pellet in 5 mL of appropriate pre-warmed medium by **gently** pipetting up and down to break up any clumps. For immediate use, dilute cells to recommended cell density in Assay Medium.
- For an overnight incubation step, plate the cells in Cell Medium in a T75 cm^2 culture flask. Incubate overnight at 37°C in a humidified atmosphere with 5% CO_2. Most cells will adhere but they will not grow, due to the gamma-irradiation process. To harvest cells, under aseptic conditions, remove media, rinse with 4.5 mL of calcium and magnesium-free PBS, add 4.5 mL Versene or calcium and magnesium-free PBS/0.5 mM EDTA, and incubate at room temperature until cells detach (do not exceed 5-10 minutes). Add 9 mL of Assay Medium, collect the cells, centrifuge (150 x g, 5 min) and resuspend in Assay Medium to the recommended cell density.

Recommended Cell Density per Assay Point (Lumilux®**):** 5 000 cells/well

- Optimal cell density per assay point will depend on the sensitivity of the reader used. The values given here are the ones determined for using the cells on a Lumilux® Reader, but may need optimization when using another reader.
- Reducing the coelenterazine concentration and loading time in the AequoScreen® assay will still result in some signal, but is expected to result in decreased signal intensity and/or stability, so we do not recommend reducing these parameters.
- Temperature should remain below 25°C during the coelenterazine loading of the cells, and until using the cells for the readings. Excessive heating by the cell stirrer for example will result in signal loss.

PerkinElmer®
For the Better

Typical Product Data



Agonist	EC_{50} (M)	% of Digitonin response
Docosahexanoic Acid	4.8×10^{-6}	85
Capric acid	3.1×10^{-5}	84

Figure 1: Agonist Response in AequoScreen® assay
An agonist dose-response experiment was performed in 384-well format using 5 000 cells/well. Luminescence was measured with the Lumilux®. Data from a representative experiment are shown. The Z'-factor was calculated for Docosahexanoic Acid with at least 16 background and 16 maximal signal points (Z'= 0.73).

PerkinElmer®
For the Better

AequoScreen® Assay Procedure (MicroBeta® JET)

Assay Buffer: DMEM / HAM's F12 with HEPES, without phenol red (Invitrogen # 11039-021) + 0.1 % protease-free BSA (from 10% solution sterilized by filtration at 0.22 µm). Store at 4°C.

Coelenterazine h: To prepare a 500 µM Coelenterazine h stock solution, solubilize 250 µg of Coelenterazine h (Promega # S2011 or Invitrogen # C6780) in 1227 µL methanol. Store at -20°C in the dark.

Digitonin: To prepare a 50 mM Digitonin stock solution, dissolve 1 g of Digitonin (Sigma # 37006) in 16.27 mL of DMSO. Aliquot and store at -20°C.

Step	Procedure
1. Cell preparation:	Resuspend thawed cells prepared as exposed above in Assay Buffer at a concentration of $3x10^5$ cells/mL.
2. Coelenterazine Loading:	Under sterile conditions, add "Coelenterazine h" at a final concentration of 5 µM to the cell suspension, mix well. Incubate at room temperature protected from light and with constant agitation for at least 4 hours (incubation can be extended overnight).
3. Cells Dilution:	Dilute cells 3x in assay buffer and incubate as described above for 60 min.
4. Ligands and plates preparation:	Prepare serial dilutions of ligands in assay buffer, (2x concentration for agonists, 3x concentration for antagonists). Dispense 50 µL of diluted ligand in a 96-well Optiplate™. ***Note: Assay can be miniaturized to 384-well and 1536-well formats.***
5. Agonist Mode Reading:	Using the reader's automatic injection system, inject 50 µL of cells (i.e. 5 000 cells) per well and immediately record relative light emission for 20-40 seconds. Digitonin at a final concentration of 50 µM in assay buffer is used in control wells to measure the receptor independent cellular calcium response.
6. Antagonist Mode Reading:	After 15 minutes of incubation of the cells with the ligand, using the reader's automatic injection system, inject 50 µL of the reference agonist at a final concentration equivalent to the EC_{80} and immediately record relative light emission for 20-40 seconds.
7. Data Analysis:	The luminescent signal is integrated from second 0 to 20-40, and the integrated value (Area Under the Curve, AUC) is used to draw sigmoidal dose-response curves.

Important Notes:

- Depending on (1) sensitivity of the reader used, (2) plate format used, and (3) assay characteristics wanted, it is possible to load cells at (a) different concentrations of cells and coelenterazine, (b) with different subsequent dilution factors, and (c) using different cell numbers per well. This is part of the validation work when importing an assay to a new reader.
- For tips and examples on running AequoScreen® assays on different readers, please refer to the AequoScreen® Starter Kit Manual available at www.perkinelmer.com/CellLines.

PerkinElmer®
For the Better

Materials and Instrumentation

The following tables provide the references of compounds and reagents used or recommended for the characterization of the human Free Fatty Acid FFA1 (GPR40) Frozen cells, as well as some advice on how to use these compounds:

Table 1. References of compounds used for functional characterization

Name	Provider	Cat n°	Working Stock Solution
Docosahexanoic Acid	Cayman	90310	Already diluted
Capric acid	Sigma	C1875	100 mM in Methanol

Table 2. References of cell culture media and assay buffers.

Note: The table below lists generic media and additives typically used for PerkinElmer Frozen cells. For product specific media and additives, please refer to the "Recommended Thawing Conditions and Handling of Frozen Cells" section.

Name	Provider	Cat n°
HAM's F-12	Hyclone	SH30026.02
DMEM	Hyclone	SH30022.02
Advanced DMEM/F12 (serotonin receptors)	Invitrogen	12634-010
EMEM	BioWitthaker	06-174G
EX-CELL DHFR$^-$ media (DHFR deficient cell lines)	Sigma	C8862
FBS	Wisent	80150
FBS dialyzed	Wisent	80950
Calcium and magnesium-free PBS	GIBCO	11010
DMEM / HAM's F12 with HEPES, without phenol red	Invitrogen	11039-021
Coelenterazine h	Promega	S2011
Coelenterazine h	Invitrogen	C6780
Digitonin	Sigma	37006
BSA, Protease-free	Sigma	A-3059
Trypsin-EDTA	Hyclone	SH30236.02
Sodium Pyruvate	GIBCO	11360
L-Glutamine	GIBCO	25030
NEAA (non-essential amino acids)	GIBCO	11140

Please visit our website: www.perkinelmer.com/CellLines for additional information on materials, microplates and instrumentation.

This product is not for resale or distribution except by authorized distributors.

LIMITED WARRANTY: PerkinElmer BioSignal Inc. warrants that, at the time of shipment, the products sold by it are free from defects in material and workmanship and conform to specifications which accompany the product. PerkinElmer BioSignal Inc. makes no other warranty, express or implied with respect to the products, including any warranty of merchantability or fitness for any particular purpose. Notification of any breach of warranty must be made within 60 days of receipt unless otherwise provided in writing by PerkinElmer BioSignal Inc. No claim shall be honored if the customer fails to notify PerkinElmer BioSignal Inc. within the period specified. The sole and exclusive remedy of the customer for any liability of PerkinElmer BioSignal Inc. of any kind including liability based upon warranty (express or implied whether contained herein or elsewhere), strict liability contract or otherwise is limited to the replacement of the goods or the refunds of the invoice price of goods. PerkinElmer BioSignal Inc. shall not in any case be liable for special, incidental or consequential damages of any kind.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

Copyright ©2009, PerkinElmer, Inc. All rights reserved. PerkinElmer® is a registered trademark of PerkinElmer, Inc. All other trademarks are the property of their respective owners.