Cellular Fatty Acid (C16) Uptake Assay Kit

Item No. 702140

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Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Size	Storage
702141	Cellular BODIPY-Palmitate Solution	1 vial/20 µl	-20°C
702142	Lipofermata Solution (40X)	1 vial/30 µl	-20°C
10009322	Cell-Based Assay Buffer Tablet	1 vial/1 ea	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

This kit may not perform as described if any reagent or procedure is replaced or modified.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. Black, clear-bottom 96-well tissue culture plate
2. Cells that will take up BODIPY-palmitate and the appropriate serum-free medium
3. A plate reader OR fluorescence microscope capable of detecting fluorescence at excitation and emission wavelengths of 488 and 508 nm, respectively
4. A source of pure water; cell culture grade, glass-distilled, or deionized water is acceptable
5. DMSO (cell culture grade)

INTRODUCTION

Background

Transport of fatty acids across the cell membrane is mediated by various membrane-associated fatty acid-binding proteins that both facilitate and regulate cellular fatty acid uptake.[1] These include membrane-associated fatty acid-binding protein ($FABP_{pm}$), CD36, also known as fatty acid translocase (FAT), and fatty acid transport proteins 1-6 (FATP1-6), which are expressed in tissues active in fatty acid metabolism.[1-3] FATP1-6 exhibit tissue-specific expression and substrate specificity, and have roles in diverse cellular processes, including thermogenesis, insulin response, bile acid synthesis, and skin homeostasis. Dysregulation of fatty acid transport, including increased transport of palmitic acid, is correlated with the development of chronic kidney disease (CKD), non-alcoholic hepatic steatosis (NASH), non-alcoholic fatty liver disease (NAFLD), obesity, and diabetes.[4-7]

Palmitic acid is a common 16-carbon saturated fat that represents 10-20% of human dietary fat intake and comprises approximately 25 and 65% of human total plasma lipids and saturated fatty acids, respectively.[8,9] Free palmitic acid can be incorporated into triglycerides or phospholipids, undergo mitochondrial beta-oxidation, or be acylated to proteins to facilitate their anchoring to biological membranes, promote protein-vesicle interactions, and regulate various G protein-coupled receptor functions.[8] This kit monitors cellular transport of a BODIPY-tagged palmitic acid, allowing for the study of a fatty acid more commonly observed in the diet than C12 fatty acids that are fluorescently tagged and often used in fatty acid transport studies.

About This Assay

Cayman's Cellular Fatty Acid (C16) Uptake Assay Kit is a convenient tool for studying cellular uptake of the fatty acid palmitate. This kit employs BODIPY-palmitate, a fluorescently tagged palmitic acid, as a probe for monitoring the uptake of fatty acid into live cells. This kit can be used to screen inhibitors of fatty acid uptake and includes lipofermata, a potent inhibitor of FATP2, as an inhibitor control. This kit includes validated protocols for kinetic uptake, endpoint uptake, and fluorescence imaging and provides enough BODIPY-palmitate for 100 tests in a 96-well format.

Principle of This Assay

This assay uses a BODIPY-tagged palmitic acid that can be utilized to monitor the kinetics of palmitate accumulation within cells or to quantify the total accumulation at discrete time points. Using a plate reader, the increase in fluorescence within cells can be monitored, and the kinetic or total measurements can be used to study how fatty acid uptake is altered by xenobiotics, disease states, genetic knockouts, or other experimental conditions.

PRE-ASSAY PREPARATION

Buffer Preparation

Cell-Based Assay Buffer

Dissolve the Cell-Based Assay Buffer Tablet (Item No. 10009322) in 100 ml of pure water. This buffer should be stable for approximately one year at room temperature and does not need to be sterile.

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

Cellular BODIPY-Palmitate Working Solution Preparation

Dilute 15 µl of Cellular BODIPY-Palmitate Solution (Item No. 702141) in 15 ml of Cell-Based Assay Buffer immediately prior to use. *NOTE: Protect from light. Cellular BODIPY-Palmitate Solution is a viscous liquid and cannot be measured by a regular pipette. A positive displacement pipette or a syringe should be used to deliver small quantities accurately.*

Lipofermata Solution (1X) Preparation

Lipofermata Solution (40X) (Item No. 702142) is provided at a concentration of 40 mM in DMSO. Dilute the Lipofermata Solution (40X) 1:10 in DMSO (cell culture grade) before diluting 1:4 in serum-free cell culture medium to a final concentration of 1,000 µM to serve as an inhibitor control.

Plate Set Up

Plate set up will depend on the specific experimental requirements of the end user. It is recommended to include a negative control that does not contain any BODIPY-palmitate to ascertain intrinsic cellular fluorescence and a control containing the provided FATP2 inhibitor lipofermata. Additionally, include appropriate vehicle controls.

Plate Reader: Kinetic Uptake

1. Culture cells in a 96-well plate under the experimental conditions required. A black, clear-bottom 96-well plate is recommended. Optimal cell numbers must be determined for each cell type, but cells should not be more than 90% confluent when assayed.
2. After cells have attached, gently remove the media and serum starve for one hour in serum-free culture media at 37°C.
3. After one hour, remove media and add 100 µl of test compound or vehicle (buffer or solvent only) in serum-free culture media. Incubate for one hour at 37°C. The test compound incubation time can be optimized based on the user's experimental needs. To use the included lipofermata as an inhibitor control, add 100 µl of the Lipofermata Solution (1X) to three wells.
4. After the test compound incubation, add 100 µl of Cellular BODIPY-Palmitate Working Solution (see page 8) to each well. Use of a multichannel pipette is recommended to ensure even loading of the probe across all samples.
5. Perform a kinetic read of the plate in a plate reader at 37°C as needed for the experiment. Readings every minute for one hour at excitation and emission wavelengths of 488 and 508 nm, respectively, is a recommended starting protocol.

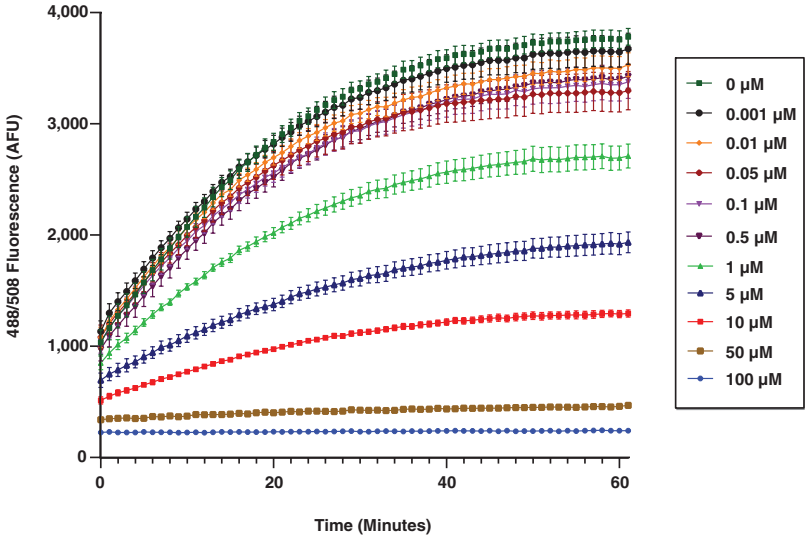

Figure 1. Kinetic uptake of BODIPY-palmitate over a one-hour period in HepG2 cells incubated with various concentrations of the FATP2 inhibitor lipofermata. Cells were seeded at a density of 20,000/well, allowed to attach overnight, and treated following described protocols.

Plate Reader: Endpoint

1. Culture cells in a 96-well plate under the experimental conditions required. A black, clear-bottom 96-well plate is recommended. Optimal cell numbers must be determined for each cell type, but cells should not be more than 90% confluent when assayed.
2. After cells have attached, gently remove the media and serum starve for one hour in serum-free culture media at 37°C.
3. After one hour, remove media and add 100 µl of the test compound or vehicle control in serum-free culture media. Incubate for one hour at 37°C. The test compound incubation time can be optimized based on the user's experimental needs. To use the included lipofermata as an inhibitor control, add 100 µl of the Lipofermata Solution (1X) to three wells.
4. After the test compound incubation, add 100 µl of Cellular BODIPY-Palmitate Working Solution (see page 8) to each well. Use of a multichannel pipette is recommended to ensure even loading of probe across all samples.
5. Incubate cells at 37°C and read the plate at excitation and emission wavelengths of 488 and 508 nm, respectively, after the appropriate incubation time has elapsed. One hour of incubation results in sufficient uptake of the probe for fluorescence to be detected *via* a conventional plate reader.

Fluorescence Microscopy

1. Culture cells in a 12- or 24-well plate under the experimental conditions required. Optimal cell numbers and density must be determined for each cell type.
2. After cells have attached, gently remove the media and serum starve for one hour in serum-free culture media at 37°C.
3. After one hour, remove media and add 500 µl (12-well plate) or 250 µl (24-well plate) of the test compound or vehicle in serum-free culture media. Incubate for one hour at 37°C. The test compound incubation time can be optimized based on the user's experimental needs. To use the included lipofermata as an inhibitor control, add 500 µl (12-well plate) or 250 µl (24-well plate) of the Lipofermata Solution (1X) to one well.
4. After the test compound incubation, add 500 µl (12-well plate) or 250 µl (24-well plate) of Cellular BODIPY-Palmitate Working Solution (see page 8) to each well.
5. Monitor fluorescence uptake of the probe using a fluorescence microscope at excitation and emission wavelengths of 488 and 508 nm, respectively.

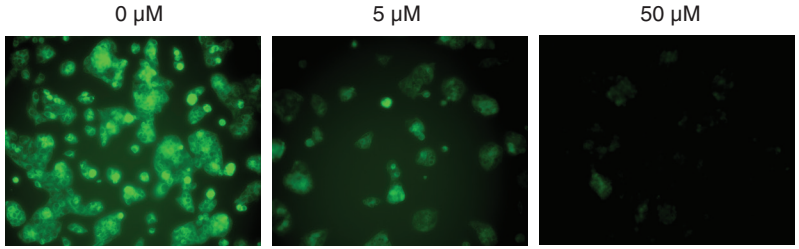

Figure 2. Fluorescence imaging of HepG2 cells following one hour of incubation with lipofermata at ex./em. of 488/508 nm.

ANALYSIS

Calculations

The IC_{50} value of a compound can be determined using a statistical software package. If performing a kinetic assay, calculate the area-under-the-curve (AUC) for each concentration tested for the entire time course of the kinetic uptake. Using the AUC, the IC_{50} value can be determined by performing a four-parameter curve fitting using a statistical analysis software. If using an endpoint read, the average final endpoint fluorescence signal for each concentration tested can be plotted against the concentration to determine the IC_{50} value of the tested compounds.

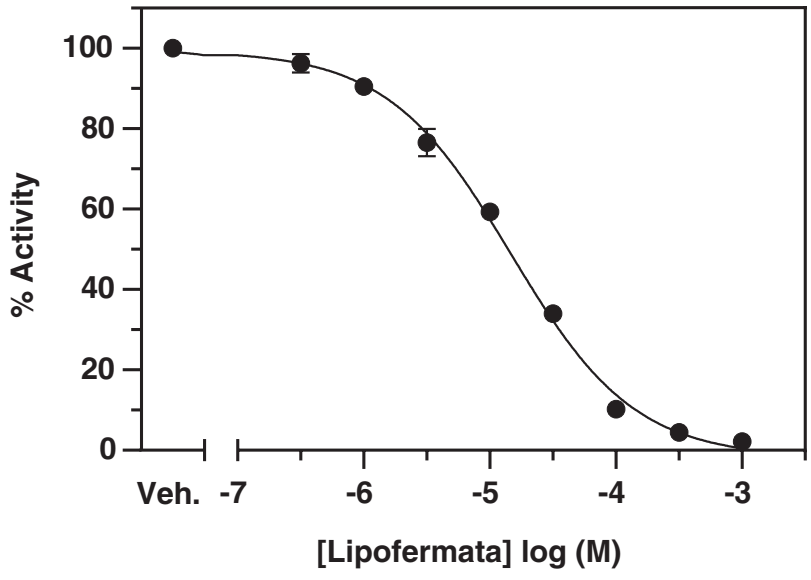

Figure 3. Inhibition of cellular palmitate uptake by lipofermata in HepG2 cells. Data are plotted as the mean of triplicate measurements +/- the standard deviation. The vehicle control (Veh.) represents 100% activity. The IC_{50} value was calculated from the final fluorescence of each well after a one-hour incubation at 37°C using a four-parameter curve fit and statistical analysis software.

Performance Characteristics

Z´ Factor:

Z´ factor is a term used to describe the robustness of an assay, which is calculated using the equation below.[10]

$$Z' = 1 - \frac{3\sigma_{c+} + 3\sigma_{c-}}{| \mu_{c+} - \mu_{c-} |}$$

Where σ: Standard deviation
μ: Mean
c+: Positive control
c-: Negative control

The theoretical upper limit for the Z´ factor is 1.0. A robust assay has a Z´ factor >0.5. The Z´ factor for Cayman's Cellular Fatty Acid (C16) Uptake Assay Kit was determined to be 0.78.

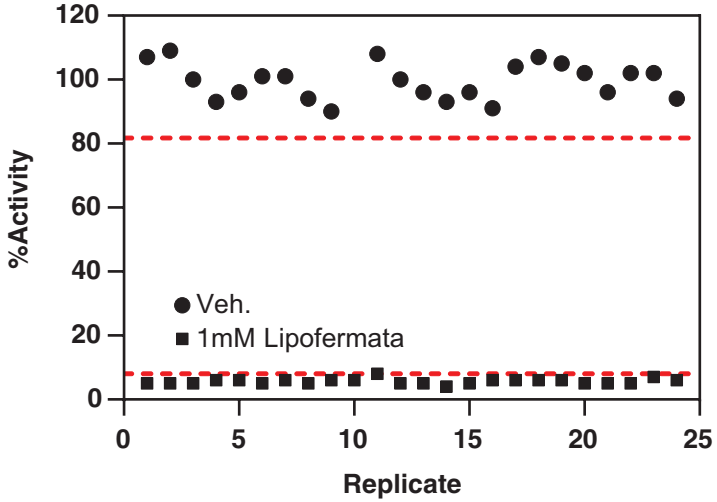

Figure 4. Typical Z' data for the Cellular Fatty Acid (C16) Uptake Assay Kit. Data are shown from 24 replicates each for the Veh., which represents 100% activity, and 1 mM lipofermata. The calculated Z' factor for this experiment was 0.78. The red dotted lines correspond to three standard deviations from the mean for each control value.

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
No BODIPY-palmitate uptake in any wells, including inhibitor control wells	Cells are not healthy	Use only healthy cells
No reduction in fluorescence intensity in negative control wells	Culture medium contains high levels of serum	Use culture medium that does not contain serum
Signal in control inhibitor wells but not in test inhibitor wells	Test inhibitor is too potent	Further optimize conditions of test inhibitors

References

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NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.