Technical Manual

Fatty Acid Uptake Assay Kit

- **Catalogue Code: BA0184**
- **Size: 100 Assays**
- **Research Use Only**

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Introduction

Long Chain Fatty Acids (LCFA) are important fuel sources for animals as substrates in β-oxidation, and serve as building blocks for many different cellular structures. Long-chain unesterified fatty acids (LCFA) are transported into cells using membrane transport proteins, and increased LCFA levels in cells are common in diabetes, obesity-related diseases, cardiovascular disease, and certain forms of cancer. Therefore, fatty acid uptake is a significant therapeutic target for the treatment of metabolic disorders and important topics for metabolic research.

How our Fatty Acid Uptake Kit Works:

Assay Genie's fluorescent cell-based fatty acid uptake assay uses a fluorescent fatty acid analog which is taken up by fatty acid transporter proteins and accumulates within the cell. Quench reagent is added to block extracellular fluorescent signal in the medium. The adherent cells import the fatty acid analog, and the bottom-read fluorimeter measures the increase in fluorescence signal at λex/em = 488/523nm. This high-throughput assay can be applied to assess fatty acid uptake activity in cells and to screen for activators and inhibitors.

Key Features:

- Safe. Non-radioactive assay.
- Fast and Sensitive. Homogenous “add-and-read” assay. No wash, lysis, or staining steps needed.
- Simple and Convenient. Can be automated as a high-throughput assay for fatty acid transport and modulator screens in cells.

Application:

Determination of long-chain fatty acid uptake in whole cells.
Evaluation of effects of ligands or drugs on fatty acid transport.

Kit Contents:

Assay Buffer: 12 mL
Substrate: 120 µL
Quench Reagent: 1 mL

MATERIALS REQUIRED BUT NOT PROVIDED

Clear bottom black cell culture 96-well plate: bottom-read fluorescence plate reader capable of reading at λex/em = 488/523 nm; Pipetting Devices

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Assay Procedure

1. To avoid cross-contamination, change pipette tips between additions of each reagent or sample. We recommend using a multi-channel pipette.

2. It is recommended that samples are assayed in triplicate or higher.

3. It is recommended to minimize the DMSO concentration of your experimental treatments, to reduce damage on the cells. Try to keep the concentration 1% or below in the wells.

A. Culture, Starve and Treat Cells

1. Plate 200 µL of 5-8 × 104

3T3-L1 cells per well in a clear-bottom black 96-well culture plate. Incubate for 4 hours or overnight at 37°C in a cell culture incubator. If cells are plated the same day as the assay, plates with adhesion-promoting polymers like poly-D-lysine are suggested.

Note: The number of cells required in a well depends on cellular size and metabolic demand of fatty acids. Different cell lines may require different cell amounts.

2. Remove the 200 µL of previous media and starve the cells in 90 µL of serum-free media for 1 hour to increase their metabolic demand.

3. Add 10 µL of the experimental treatment to the starvation media and incubate at desired temperature and for desired length of time (example: 37°C for 30 minutes).

Make sure to include a control group with 10 µL of the same solvent that the treatment is dissolved in, such as 10% DMSO, and a blank well with just media and no cells.

B. Add Working Reagent

1. Working Reagent Preparation: Make the Working Reagent 10 minutes and warm to 37°C before adding to the wells. For each reaction well, prepare Working Reagent by mixing 1 µL of Substrate, 8 µL of Quench

Reagent, and 100 µL of Assay Buffer.

2. Leaving the serum-free media in the well, add an additional 100 µL of Working Reagent to the cells.

3. Read the plate at λex/em = 488/523 nm for 60 minutes at 37°C. The plate should be using bottom-read settings. Use data from 60 minutes (F60).

Note: If these wavelengths are not available, excitation can vary from 470-495, and emission can vary from 500 to 550 nm. Fluorescence may be lower at these settings. It is recommended to run kinetic data and measure every 1-5 minutes if possible.

Inhibitor Screening protocol (example):

1. Plate cells, and incubate for at least 4 hours at 37°C. Remove cell culture media, and replace with 90 µL of serum-free media for 1 hour.

2. Prepare inhibitor concentrations at 10X the final concentration, and add 10 µL to the wells so that the compound is 1X concentration in the well. As a control, add 10 µL of the solvent that the test compounds are dissolved in (such as 10% DMSO) to your control wells. Incubate for 30 minutes at 37°C.

4. Add the working reagent with a multichannel pipettor, and read the plate at λex/em = 488/523 nm for 60 minutes at 37°C.

5. Calculate % activity using the equation below.

Optional: After screening for inhibition, it may be helpful to screen for cytotoxicity to remove compounds which seem to inhibit fatty acid uptake but instead kill the cells.

Calculations

Compute the mean Fluorescence for each treatment by averaging the replicates for each group. The change in activity is calculated as follows:

$$\text{Fatty Acid Uptake Activity (\%)} = \frac{Ftreated - Fblank}{Fcontrol - Fblank}\ x\ 100$$

$F_{Treated}$ is the mean F of the cells that have been dosed with an experimental compound, $F_{Control}$ is the mean F of the cells that have not been dosed, and F_{Blank} is the mean F of blank (medium only) wells with no cells.

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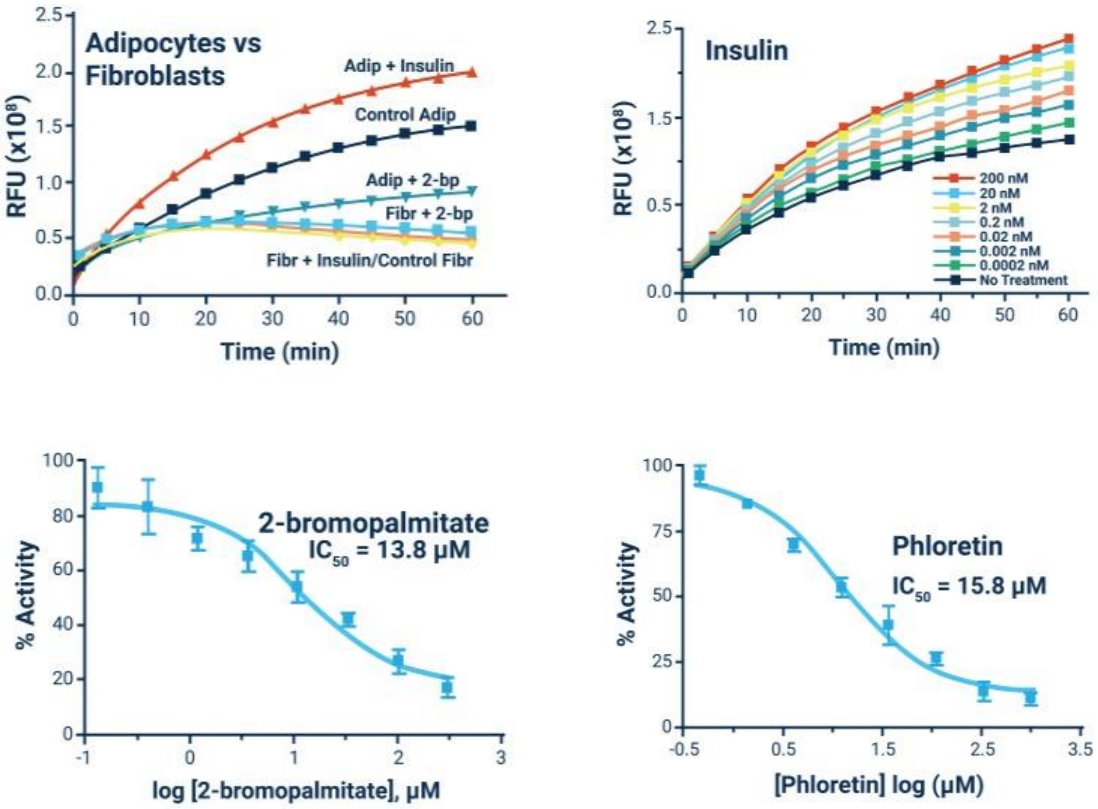

Fatty Acid Uptake Assay in 3T3-L1 Cells.

3T3 L-1 cells were seeded at 60,000 cells/well, starved, and treated according to protocol. Top Left: Differentiated and undifferentiated 3T3-L1 activity in the absence and presence of 160 nM insulin, and 30 µM competitor 2-bromopalmitate. Top Right: Increase in fatty acid uptake of 3T3-L1 adipocytes from insulin. Bottom Left: Fatty Acid Transport Inhibition curve of 2-bromopalmitate. Inhibitor was added alongside working reagent, instead of during a 30 minute preincubation. Bottom Right: Fatty Acid Transport Inhibition curve of Phloretin. Inhibitor was incubated for 30 minutes prior to run

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