Cell Meter™ Fluorimetric Fatty Acid Uptake Assay Kit

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 36385 (100 tests)	Keep in freezer Avoid exposure to light	Fluorescence microscope Fluorescent microplate readers

Introduction

Fatty acid uptake is an important therapeutic target for the treatment of many human diseases such as obesity, type 2 diabete and hepatic steatosis. The ScreenQuest™ Fluorimetric Fatty Acid Uptake Assay Kit provides a simple and sensitive method for the measurement of fatty acid uptake in cells containing fatty acid transporters. The kit uses a proprietary dodecanoic acid fluorescent fatty acid substrate. This fatty acid uptake assay kit can be performed on any fluorescence microplate reader with a bottom-read mode at Ex/Em = 485/515 nm or FITC channel. The assay can be performed in 96-well or 384-well microtiter plates in a simple mix-and-read procedure, and easily adapted for high throughput screening applications.

Kit Components

Components	**Amount**
Component A: TF2-C12 Fatty Acid	1 vial, lyophilized
Component B: Assay Buffer	10 mL
Component C: DMSO	100 µL

Assay Protocol for one 96-well plate

Brief Summary

Plate cells in growth medium for 4-6 hours → Deprive the cells for 1 hour, and treat the cells as desired→ Add 100µl/well of the fatty acid dye-loading solution → Monitor fluorescence increase at Ex/Em = 485/515 nm immediately for kinetics or after 60 minutes incubation for endpoint reading (bottom read mode)

1. Prepare Cells:

Prepare cells as desired. The following protocols are guidelines to prepare 3T3-L1adipocytes.

1.1 Prepare differentiated 3T3-L1 adipocytes (see Ref 1): 3T3-L1 fibroblasts were grown 2 days in a 75 cm flask post-confluence in DMEM/FBS, and then for 2 days in DMEM/FBS supplemented with 0.83µM insulin, 0.25µM dexamethasone, and 0.25mM isobutylmethylxanthine. The medium is changed to maintain the insulin concentration with dexamethasone and IBMX absent for another 2 days. The medium was then changed to DMEM/FBS alone for another 3-5 days. Differentiated cells (at least 95% of which showed an adipocyte phenotype by accumulation of lipid droplets) were used on day 8 to 12 after induction of differentiation.

1.2 Plate 3T3-L1 adipocytes in growth medium at 50,000-80,000 cells/well/100µl/96-well or 12,500-20,000 cells/well/25µl/384-well black wall/clear bottom cell culture Poly-D lysine plate for 4-6 hours before experiment. Centrifuge the plate at 800 rpm for 2 minutes with brake off.

Note 1: It is recommended to plate 3 wells with growth medium only (without cells) as blank wells for data normalization; Note 2: We find that adipocytes plated at the same day (4-6 hours, and then serum deprived for 1 hour) give better results than that plated for overnight.

1.3 Remove the cell plate from the incubator, aspirate the medium from the wells, and deprive the cells with 90 µl/well/ 96 well-plate or 20 µl /well/384 well-plate serum free medium. Incubate the cells at 37 ºC, 5% CO_2 incubator for 1 hr.

1.4 Treat the cells by adding 10µl/well/96-well plate (5µl/well/384-well plate) of the test compounds or 1X Hanks and 20 mM Hepes buffer (1X HBSS, pH 7.4) or buffer of your choice as the compound diluent. For blank wells, add the compound diluents. Incubate the cells at 37 ºC, 5% CO_2 incubator for a desired period of time (30 minutes for 3T3-L1 cells treated with Insulin).

2. Prepare fatty acid dye-loading solution:

2.1 Thaw all the kit components at room temperature before use.

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Technical Support: support@aatbio.com; Tel: 408-733-1055

2.2 Make TF2-C12 Fatty Acid stock solution: Add 20 µL DMSO (Component C) into the vial of the TF2-C12 Fatty Acid (Component A), and mix them well.
Note: 20 µL of the fluorescent fatty acid substrate stock solution is enough for one plate. The unused fluorescent fatty acid substrate stock solution can be aliquoted and stored at ≤ -20 °C for up to two months if the tubes are sealed tightly and kept from light. Avoid repeated freeze-thaw cycles.

2.3 Make fatty acid dye-loading solution: Add 20 µL of the TF2-C12 Fatty Acid stock solution (from Step 2.2) to 10 mL of Assay Buffer (Component B), and mix them well.
Note1: 10 mL of fatty acid dye-loading solution is enough for one plate, prepare fresh for each experiment.

3. Run Fatty Acid Uptake Assay:

3.1 Remove compound-treated cell plates from the incubator (from Step1.4), add 100µl/well/96-well plate or 25µl/well/384-well plate (including blank wells) of the fatty acid dye-loading solution (from Step 2.3).

3.2 Measure the fluorescence signal with a fluorescence microplate reader at Ex/Em = 485/515 nm (cut off at 495 nm) using a bottom read mode.
For kinetic reading: Read the fluorescence intensity immediately at 20 seconds interval for 30-60 minutes.
For endpoint reading: Read the fluorescence intensity at the end of the 30-60 minutes incubation.

Data Analysis

The fluorescence in blank wells with the growth medium is subtracted from the values for the wells with cells. The background fluorescence of the blank wells may vary depending on the sources of the growth media or the microtiter plates.

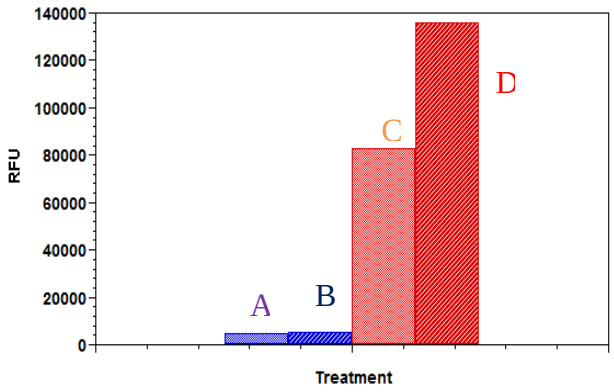

Figure 1. Comparison of fatty acid uptake by 3T3-L1 adipocytes and fibroblast. Cells were plated at 50,000 cells/100 µL/well in a 96 well black wall/clear bottom poly-D lysine plate for 5 hours, and then serum deprived for 1 hour. Cells were treated without (control) or with insulin (150 nM), and incubated at 37 ºC, 5% CO_2 incubator for 30 min. At the end of the incubation time, 100 µl of fatty acid mixture was added into the well, and incubated for another 60 min, the fluorescence signal was measured with a FlexStation plate reader using bottom read mode. A – fibroblasts (Control); B – fibroblasts (Insulin); C – adipocytes (Control); D– adipocytes (Insulin).

References

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