Screen Quest™ Fluorimetric Long Chain Fatty Acid Uptake Assay Kit

Catalog number: 36386
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 36386)
Component A: TF2-C16 Fatty Acid	Freeze (< -15 °C), Minimize light exposure	1 vial, lyophilized
Component B: Assay Buffer	Freeze (< -15 °C)	10 mL
Component C: DMSO	Refrigerated (2-8 °C)	100 µL

OVERVIEW

Screen Quest™ Fluorimetric Long Chain Fatty Acid Uptake Assay Kit provides a convenient method for studying cellular uptake of long-chain fatty acids in live cells. The assay employs a fluorescently labeled C16 fatty acid analog to monitor fatty acid transport activity mediated by membrane-associated fatty acid transport proteins and related uptake pathways. Dysregulation of long-chain fatty acid uptake is associated with metabolic disorders including obesity, hepatic steatosis, and type 2 diabetes. The assay supports kinetic uptake measurements, endpoint analysis, and fluorescence imaging using standard fluorescence microplate readers equipped with bottom-read capability or FITC filter settings. The streamlined workflow is compatible with both 96-well and 384-well microplate formats, making the kit suitable for routine cellular fatty acid uptake studies as well as high-throughput screening applications.

AT A GLANCE

Protocol Summary

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

Important Note

Thaw all the kit components at room temperature before starting the experiment.

CELL PREPARATION

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

- **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 0.83 µM insulin, 0.25 µM dexamethasone, and 0.25 mM 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.
- Plate 3T3-L1 adipocytes in growth medium at 50,000-80,000 cells/well/100 µL/96-well or 12,500-20,000 cells/well/25 µL/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: It is recommended to plate 3 wells with growth medium only (without cells) as blank wells for data normalization.

  Note: 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.
- 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 wellplate serum free medium. Incubate the cells at 37 °C, 5% CO2 incubator for 1 hr.
- Treat the cells by adding 10 µL/well/96-well plate (5 µL/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 °C, 5% CO2 incubator for a desired period of time (30 minutes for 3T3-L1 cells treated with Insulin).

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles. Thaw all the kit components at room temperature before use.

TF2-C16 Fatty Acid Stock Solution

Add 20 µL of DMSO (Component C) to the vial of TF2-C16 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.

PREPARATION OF WORKING SOLUTION

Fatty Acid dye-loading solution

Add 20 µL of the TF2-C16 Fatty Acid stock solution to 10 mL of Assay Buffer (Component B) and mix them well.

Note: 10 mL of Fatty Acid dye-loading solution is enough for one plate; prepare fresh for each experiment.

SAMPLE EXPERIMENTAL PROTOCOL

1. Treat cells with test compounds as desired.
2. Remove compound-treated cell plates from the incubator, add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) (including blank wells) of the Fatty Acid dye-loading solution.
3. 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.

Note: For kinetic reading: Read the fluorescence intensity immediately at 20 seconds interval for 30-60 minutes.

Note: For endpoint reading: Read the fluorescence intensity at the end of the 30-60 minutes incubation.

EXAMPLE DATA ANALYSIS AND FIGURES

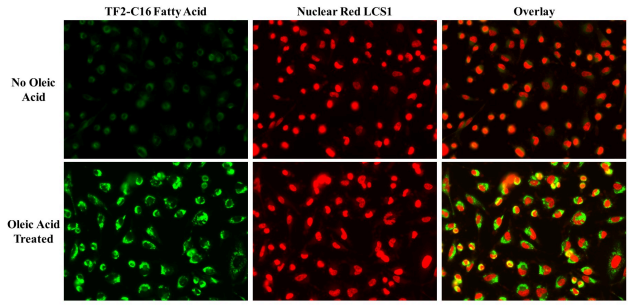

Figure 1. Fluorescence images of intracellular lipid droplets in control (top) and Oleic Acid treated HeLa cells (bottomRight) using Screen Quest™ Fluorimetric Long Chain Fatty Acid Uptake Assay Kit. HeLa cells were incubated with 100 µM of Oleic Acid for 24 hours. After washing with PBS, the cells were incubated with TF2-C16 Fatty Acid for 60 minutes. Cells were co-stained with Nuclear Red LCS1. Image was acquired with FITC (lipid uptake) and Cy5 (nuclear counter staining) filter set.

DISCLAIMER

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