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Product Information

Fatty Acid Uptake Kit

Catalog Number **MAK156**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description
Fatty acids are critical for many biological processes including energy metabolism and the synthesis of structural cellular components. The facilitated uptake of fatty acids, presumably through fatty acid transport proteins, is thought to be regulated at multiple levels. The dysregulation of fatty acid uptake may contribute to diseases such as obesity, type 2 diabetes, and hepatic steatosis.

This kit is provides a simple and sensitive method for the measurement of fatty acid uptake in cells containing fatty acid transporters. This kit uses a proprietary dodecanoic acid fluorescent fatty acid substrate whose uptake into cells result in an increase in fluorescence intensity (λ_{ex} = 485/λ_{em} = 515 nm).

Components
The kit is sufficient for 100 assays .

TF2-C12 Fatty Acid Catalog Number MAK156A	1 vl
Assay Buffer Catalog Number MAK156B	10 mL
DMSO Catalog Number MAK156C	0.1 mL

Reagents and Equipment Required but Not Provided.
- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorometric assays.
- Fluorescence multiwell plate reader or fluorescence microscope

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
The kit is shipped under ambient conditions and storage at –20 °C, protected from light, is recommended.

Procedure
Allow all reagents to come to room temperature before use. Briefly centrifuge vials before opening.

Preparation of Cells

1. Prepare cells as desired. For example, differentiate 3T3-L1 cells to 3T3-L1 adipocytes using desired protocol.

2. Plate cells in growth medium at 50,000 to 80,000 cells/well/90 µL for a 96 well plate or 12,500–20,000 cells/well/20 µL for a 384 well plate for 4–6 hours before experiment. If desired, cells can be plated on poly-D-Lysine coated plates. Centrifuge the plate at 800 rpm for 2 minutes with brake off.
   Note: It is recommended to plate 2–3 wells with growth medium only (no cells) to serve as blank wells for data normalization. The length of time cells should be plated before experiment may need to be optimized.

3. Remove the plate from the incubator and aspirate the medium from the wells. Add 90 µL/well for 96 well plate or 20 µL/well for 384 well plate of serum-free medium. Incubate the cells at 5% CO_2, 37 °C for 1 hour.

4. Treat the cells with 10 µL/well for 96 well plate or 5 µL/well for 384 well plate of test compounds and suitable controls. Add diluent only to blank wells. Incubate the cells at 5% CO_2, 37 °C for desired period of time.

Assay Procedure

Allow all reagents to come to room temperature before use. Briefly centrifuge vials before opening.

1. Prepare the TF2-C12 Fatty Acid Stock Solution by adding 20 µL of DMSO into the TF2-C12 Fatty Acid vial and mixing well by pipetting.

2. Add 20 µL of the TF2-C12 Fatty Acid Stock Solution to 10 mL of Assay Buffer to prepare the Fatty Acid Dye Loading Solution. This will prepare enough Fatty Acid Dye Loading Solution for one plate. If less Dye Loading Solution is needed, the amount of prepared solution can be scaled down and remaining TF2-C12 Fatty Acid Stock Solution can be stored in aliquots at –20 °C for up to two months. Do not store prepared Dye Loading Solution. Dye Loading Solution should be prepared fresh for each experiment.

3. Remove cell plate from incubator and add 100 µL/well for 96 well plate or 50 µL/well for 384 well plate of Fatty Acid Dye Loading Solution to each of the sample, blank, and control wells.

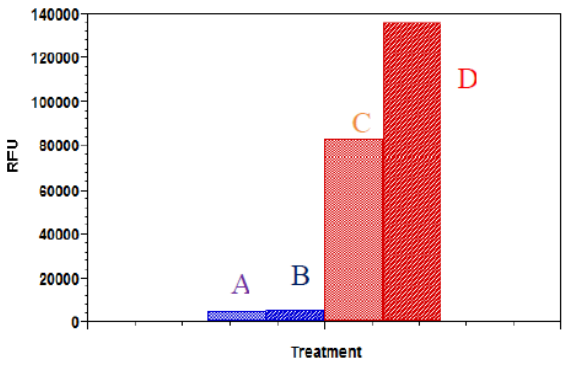
4. Take the initial measurement of fluorescence intensity (λ_{ex} = 485/λ_{em} = 515 nm). Continue to measure the flouorescence every 20 seconds for 30–60 minutes or measure after a 30–60 minute incubation.

Results

Correct for the background by subtracting the values obtained for the blank wells from the values obtained from the samples.

Figure 1.

Comparison of fatty acid uptake by 3T3-L1 adipocytes and fibroblasts



Cells were plated at 50,000 cells/100 µL/well for 5 hours and then serum-starved for 1 hour. Cells were treated with medium only (control) or insulin (150 nM), and incubated at 5% CO_2, 37 °C for 30 minutes. Following the incubation, 100 µL of Fatty Acid Dye Loading Solution was added to each well and incubated for an additional 60 minutes.

A – Fibroblasts with medium only
B – Fibroblasts treated with insulin
C – Adipocytes with medium only
D – Adipocytes with insulin

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