# ab176768

# Free Fatty Acid Uptake Assay Kit (Fluorometric)

## Instructions for Use

For measurement of fatty acid uptake in cells containing fatty acid transporters.

This product is for research use only and is not intended for diagnostic use.

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# Table of Contents

# 1. Overview

Fatty acid uptake is an important therapeutic target for the treatment of many human diseases such as obesity, type 2 diabetes and hepatic steatosis. Abcam's Free Fatty Acid Uptake Assay Kit (Fluorometric) (ab176768) 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.

## 2. Protocol Summary

Plate cells in growth medium for 4-6 hours

Deprive the cells for 1 hour, and treat the cells as desired

Add 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)

## 3. Kit Components

| Item | Quantity | Storage upon arrival | Storage after use/ reconstitution |
|---|---|---|---|
| TF2-C12 Fatty Acid | 1 vial | -20°C | -20°C |
| Assay Buffer | 10 mL | -20°C | -20°C |
| DMSO | 100 µL | -20°C | -20°C |

## 4. Storage and Stability

Upon arrival, store the kit at-20°C and protected from light. Please read the entire protocol before performing the assay. Avoid repeated freeze/thaw cycles.

Warm all buffers to room temperature before use. Briefly centrifuge all small vials prior to opening.

## 5. Materials Required, Not Supplied

- 96 or 384-well black plate with clear flat bottoms
- 75cm flask
- Serum free medium
- Fluorescence microplate reader

# 6. Assay Protocol

**1. Reagent Preparation**

Thaw all the kit components at room temperature before use.

**a) TF2-C12 Fatty Acid stock solution:**

Add 20 µL DMSO into the vial of the TF2-C12 Fatty Acid, 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.*

**b) Fatty acid dye-loading solution:**

Add 20 µL of the TF2-C12 Fatty Acid stock solution (from Step 1a) to 10 mL of Assay Buffer, and mix them well.

*Note: 10 mL of fatty acid dye-loading solution is enough for one plate, prepare fresh for each experiment.*

**2. Prepare cells**

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

**a)** Prepare differentiated 3T3-L1 adipocytes: 3T3-L1 cells were grown 2 days in a 75 cm flask post-confluence in

DMEM/FBS, and then for 2 days more 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.

**b)** Plate 3T3-L1 adipocytes in growth medium at $5*10^4$ – $8\text{-}10^4$ cells/well/100μL/ 96-well or 12,500-20,000 cells/well/25μL/ 384-well black wall or 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.*

**c)** Remove the cell plate from the incubator, aspirate the medium from the wells, and deprive the cells with 90μL/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.

**d)** 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 37ºC, 5% $CO_2$ incubator for a desired period of time (30 minutes for 3T3-L1 cells treated with Insulin).

**3. Run Fatty Acid Uptake assay**

**a)** Remove compound-treated cell plates from the incubator (from Step 2d)' add 100µL/well/96-well plate or 25µL/well/384-well plate (including blank wells) of the fatty acid dye-loading solution (from Step 1b).

**b)** 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.

# 7. Data Analysis

**Calculations:**

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.

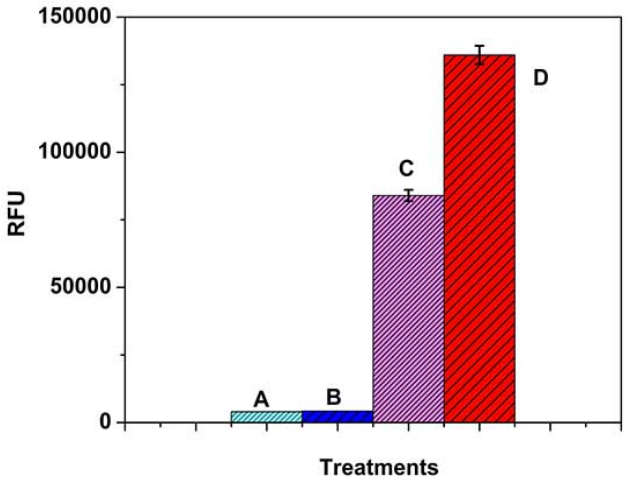


**Figure 1.** Comparison of fatty acid uptake by 3T3-L1 adipocytes and fibroblast. Cells were plated at 50,000 cells/100 µL/well in a 96 well black wall/clear bottom poly-D lysine plate for 5 hours, and then

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 plate reader using bottom read mode. A – fibroblasts (Control); B – fibroblasts (Insulin); C – adipocytes (Control); D– adipocytes (Insulin).

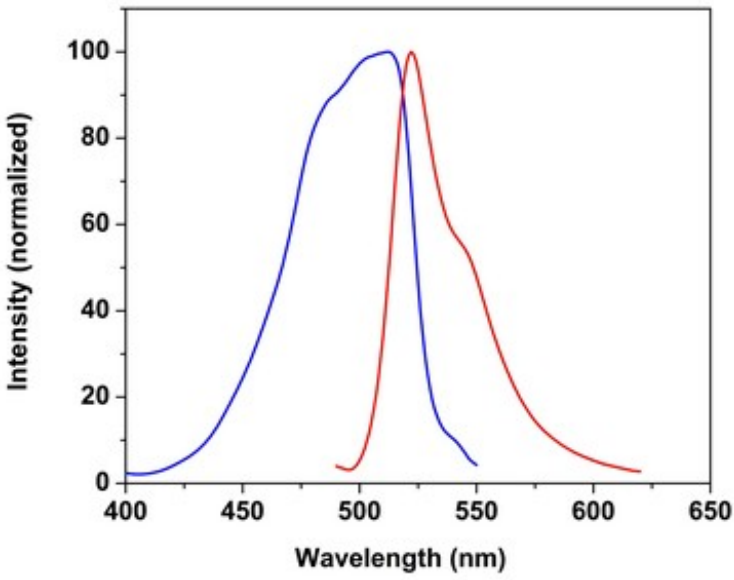


**Figure 2.** Excitation and emission spectra of Free Fatty Acid Uptake Assay Kit (Fluorometric) (ab176768).

# 8. Troubleshooting

| Problem | Reason | Solution |
|---|---|---|
| Assay not working | Assay buffer at wrong temperature | Assay buffer must not be chilled - needs to be at RT |
| | Protocol step missed | Re-read and follow the protocol exactly |
| | Plate read at incorrect wavelength | Ensure you are using appropriate reader and filter settings (refer to datasheet) |
| | Unsuitable microtiter plate for assay | Fluorescence: Black plates (clear bottoms);<br>Luminescence: White plates;<br>Colorimetry: Clear plates.<br>If critical, datasheet will indicate whether to use flat- or U-shaped wells |
| Unexpected results | Measured at wrong wavelength | Use appropriate reader and filter settings described in datasheet |
| | Samples contain impeding substances | Troubleshoot and also consider deproteinizing samples |
| | Unsuitable sample type | Use recommended samples types as listed on the datasheet |
| | Sample readings are outside linear range | Concentrate/ dilute samples to be in linear range |

| Problem | Reason | Solution |
|---|---|---|
| Samples with inconsistent readings | Unsuitable sample type | Refer to datasheet for details about incompatible samples |
| | Samples prepared in the wrong buffer | Use the assay buffer provided (or refer to datasheet for instructions) |
| | Samples not deproteinized (if indicated on datasheet) | Use the ***10kDa spin column (ab93349)*** |
| | Cell/ tissue samples not sufficiently homogenized | Increase sonication time/ number of strokes with the Dounce homogenizer |
| | Too many freeze-thaw cycles | Aliquot samples to reduce the number of freeze-thaw cycles |
| | Samples contain impeding substances | Troubleshoot and also consider deproteinizing samples |
| | Samples are too old or incorrectly stored | Use freshly made samples and store at recommended temperature until use |
| Lower/ Higher readings in samples and standards | Not fully thawed kit components | Wait for components to thaw completely and gently mix prior use |
| | Out-of-date kit or incorrectly stored reagents | Always check expiry date and store kit components as recommended on the datasheet |
| | Reagents sitting for extended periods on ice | Try to prepare a fresh reaction mix prior to each use |
| | Incorrect incubation time/ temperature | Refer to datasheet for recommended incubation time and/ or temperature |
| | Incorrect amounts used | Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume) |

| Problem | Reason | Solution |
|---|---|---|
| Standard curve is not linear | Not fully thawed kit components | Wait for components to thaw completely and gently mix prior use |
| | Pipetting errors when setting up the standard curve | Try not to pipette too small volumes |
| | Incorrect pipetting when preparing the reaction mix | Always prepare a master mix |
| | Air bubbles in wells | Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates |
| | Concentration of standard stock incorrect | Recheck datasheet for recommended concentrations of standard stocks |
| | Errors in standard curve calculations | Refer to datasheet and re-check the calculations |
| | Use of other reagents than those provided with the kit | Use fresh components from the same kit |

**Notes**

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