

ab287857 – Fatty Acid Uptake Assay Kit

For the characterization of fatty acid uptake kinetics in live cells, and the screening and characterization of drugs and test compounds for modulation of fatty acid uptake
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab287857>

Materials Supplied

Item	Quantity	Storage Condition
FA Uptake Assay Buffer	25 mL	-20°C
100X Extracellular Quenching Solution	200 µL	-20°C
200X Fluorescent Fatty Acid Probe	100 µL	-20°C

Materials required but not supplied

- Cell line for testing: cells that express membrane fatty acid transporter proteins (differentiated 3T3-L1 adipocytes or heterologous cells stably transfected with desired FATP(s))
- Serum-free/phenol red-free cell culture medium and 5% CO₂ cell culture incubator
- Multiwell fluorescence microplate reader (capable of bottom read)
- Black-walled 96-well tissue-culture plates with clear flat bottom wells

Storage Conditions and Reagent Preparation:

- Store kit at -20°C and protect from light. Briefly centrifuge all small vials prior to opening. Open all of the reagents under sterile conditions (e.g. a cell culture hood) only.
- Read entire protocol before performing the assay procedure.

FA Uptake Assay Buffer: Allow to thaw to room temperature and open under sterile conditions. Store at -20°C.

100X Extracellular Quenching Solution: Aliquot the stock solution as desired and store aliquots at -20°C, protected from light. Stable for at least 4 freeze/thaw cycles.

200X Fluorescent Fatty Acid Probe: Aliquot the stock solution as desired and store aliquots at -20°C, protected from light. Avoid repeated freeze/thaw cycles.

Fatty Acid Uptake Assay Protocol:

The procedure described below employs differentiated 3T3-L1 adipocytes as a model cell line for measuring fatty acid uptake. Undifferentiated 3T3-L1 cells (ATCC CL-173) display a fibroblast-like morphology but become rounded and accumulate lipid droplets several days after the initiation of differentiation. Primary adipocytes derived from tissues or other cell lines known to import long-chain fatty acids (such as Caco-2 or HepG2 cells) may also be used if desired.

1. 3T3-L1 Adipocyte Differentiation and Seeding:

- Grow 3T3-L1 fibroblasts in preadipocyte growth medium (DMEM with 10% BCS) until cells are maximally confluent (>95%). At two days post-confluence, replace preadipocyte medium with differentiation medium (DMEM/F12 with 10% FBS) containing a cocktail of induction agents. Incubate cells for 3 days at 37°C in a humidified cell culture incubator with a 5% CO₂ atmosphere, then replace differentiation medium with maintenance medium (DMEM/F12 with 10% FBS, supplemented with 1.5 µg/ml insulin). Change maintenance medium every 2-3 days, until at least 90% of the cells exhibit an adipocyte-like morphology, with intracellular lipid droplets visible by brightfield microscopy (typically 7-10 days after initiation of differentiation). Once 3T3-L1 adipocytes are fully differentiated, the

insulin-supplemented medium should be replaced with standard DMEM/F12 with 10% FBS.

- Trypsinize 3T3-L1 adipocytes the night prior to assay and seed approximately 7-8 x 10⁴ cells/well in an black-walled 96-well tissue culture plate (with flat, clear-bottom wells) using 200 µl culture media (DMEM/F12 with 10% FBS) per well. Grow cells overnight in a 5% CO₂ atmosphere 37°C incubator (adherent cell monolayer should be ~80-90% confluent for optimal assay).

Δ Notes:

- Differentiated 3T3-L1 adipocytes are easily ruptured in response to mechanical shearing during trypsinization and seeding. For best results, handle cells gently and avoid excessive mechanical agitation or intense, vigorous pipetting force.
- If desired, 3T3-L1 adipocytes may be seeded in the 96-well plate on the day of the fatty acid uptake assay rather than the night before. Seed 7-8 x 10⁴ cells per well in 200 µl culture media and incubate at 37°C in 5% CO₂ atmosphere for 5 hours. If seeding cells on the day of the assay, we recommend using plates coated with an adhesion-promoting polymer such as poly-D-lysine.

2. Serum Starvation and Uptake Assay Reaction Preparation:

- Warm FA Uptake Assay Buffer to 37°C. Remove plate from the incubator. Prepare background control (no-cells) well(s) by adding 90 µl of serum-free, phenol red-free medium to blank well(s). For sample wells (with cells), gently aspirate growth medium from wells and replace with 90 µl of serum-free, phenol red-free medium. Return cells to CO₂ incubator and incubate at 37°C for 1 hour.

Δ Note: To minimize the loss of the cells during medium exchange, we recommend centrifuging the plate in a 96-well plate-compatible centrifuge at 800 x g for 3 min with the brake function turned off prior to aspiration of growth medium.

- Dissolve fatty acid uptake-modulating test compounds in proper solvent(s) to produce stock solutions. For each test compound, prepare a 10X working solution of each desired test concentration by diluting stock solutions in serum-free, phenol red-free medium. To determine IC₅₀ / EC₅₀ values for test compounds, 10X working solutions should be prepared in a range of concentrations in order to generate a multi-point dose-response curve. If an organic solvent is used to dissolve test compounds, the concentration of organic solvent should be the same for all test compound dilutions.
- Following serum starvation, remove cells from incubator and treat cells by adding 10 µl of either 10X test compound working solution to designated wells. For vehicle control and blank wells, add 10 µl of serum-free, phenol red-free medium (with the same final concentration of organic solvent used to solubilize test ligands, if applicable). Return plate to 5% CO₂ atmosphere incubator and incubate treated cells at 37°C for 30 min (or desired time based upon your specific treatment conditions and protocol).
- Prepare a 2X solution of quenched Uptake Reaction Mix by adding 200 µl of the 100X Extracellular Quenching Solution stock and 100 µl of the 200X Fluorescent Fatty Acid Probe to 9.7 ml pre-warmed FA Uptake Assay Buffer. This preparation yields 10 ml of 2X Uptake Reaction Mix, which is sufficient for 100 reaction wells, but can be scaled depending upon the number of reactions to be performed. Keep the 2X Uptake Reaction Mix protected from light and warmed at 37°C until use.

Measurement:

Remove plate from cell culture incubator and transfer to microplate reader. Add 100 µl of prewarmed 2X Uptake Reaction Mix (see step 2d) to all wells, including blank wells (for a final volume of 200 µl per well). Immediately begin measuring the fluorescence (Ex/Em = 488/523 nm) of all of the wells in kinetic mode at 37°C for 60 min using the 'bottom read' function.

Δ Note: While we recommend reading in kinetic mode, the assay may also be performed in endpoint mode. Following addition of Uptake Reaction Mix, incubate the plate at 37°C for 60 min (protected from light), then measure endpoint fluorescence in bottom-read mode.

Calculation:

For each test compound or treatment condition, quantify the relative stimulation/inhibition of fatty acid uptake versus vehicle control using the equation below, where RFU_{vehicle} is the fluorescence intensity of the vehicle control condition, RFU_{treatment} is the fluorescence intensity of the treated well and RFU_{blank} is the fluorescence intensity of the background control (no cells) well:

$$\% \text{ Activity} = (RFU_{\text{treatment}} - RFU_{\text{blank}}) / (RFU_{\text{vehicle}} - RFU_{\text{blank}}) \times 100$$

Technical Support

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