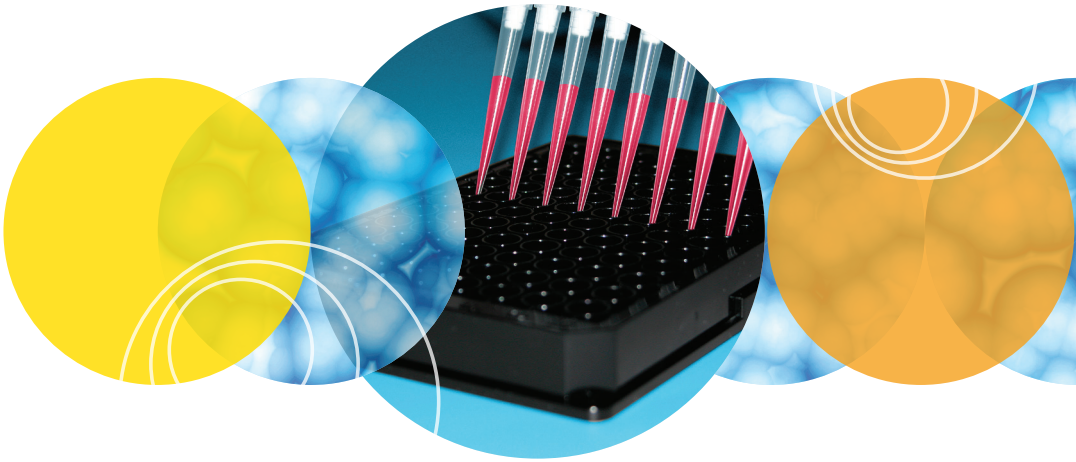


QBT Fatty Acid Uptake Assay Kit

A kinetic uptake assay in a homogeneous fluorescent format

KEY FEATURES

- **Homogeneous, live-cell fluorescent assay**
- **Convenient, single reagent addition assay format**
- **No use of radioactivity required**
- **Amenable to automation and HTS**
- **Analyze on any bottom-read fluorescent microplate instrument**
- **Read in endpoint mode for high-throughput applications**
- **Read in real-time kinetic mode for mechanistic studies**

Fatty acid uptake and transporters

Fatty acids are utilized for diverse cellular processes including mitochondria oxidation, membrane synthesis and energy storage. Pathologically increased intracellular fatty acid concentrations can cause cellular apoptosis and have been linked to insulin desensitization, Type 2 diabetes, obesity and cardiovascular disease. All of these intracellular processes are dependent upon fatty acids traversing the plasma membrane to get into the cell. Therefore, understanding the Fatty Acid Transport Proteins (FATP) that regulate this process is of great importance in biomedical research and drug discovery.

FATPs are part of an evolutionarily conserved family of proteins of which six FATP family members are expressed in the human genome. While all six human FATPs are similar in function, they differ dramatically in their expression in various tissue and organs. The differences in FATP expression pattern therefore make it possible to selectively target the uptake of fatty acids into specific organs by using compounds that can discriminate between the different FATP family members.

Conventional methods

The development of fatty acid uptake regulators as potential drugs for metabolic diseases requires robust assays for the quantitation of fatty acid uptake. Conventional protocols utilizing radioactivity often require cell lysis and processing at a very low temperature, making them expensive, slow and not suitable for high throughput screening. Fluorescence-based protocols generally require the use of low throughput Fluorescence Activated Cell Sorter (FACS) instrumentation, or require cell washing, which can severely compromise the integrity of fragile adipocyte cells.

MOLECULAR DEVICES

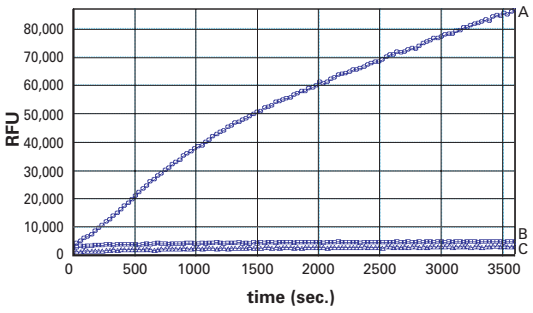


Figure 1. Fibroblasts vs. adipocytes. 3T3 L1 adipocytes were plated at 50,000 cells/well in 100 µL of DMEM/FBS in a 96-well plate and incubated at 37°C for five hours. Growth medium was then replaced with fatty acid uptake solution. Kinetic readings were started immediately with a Molecular Devices FlexStation Plate Reader. **A:** adipocytes; **B:** fibroblasts; **C:** no cells (uptake reagent only).

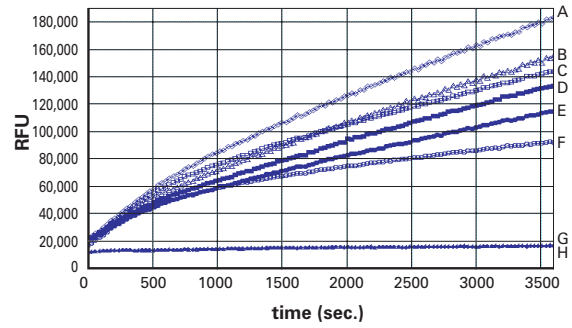


Figure 2. Fibroblasts vs. adipocytes: insulin dose-response. 3T3 L1 adipocytes were plated at 50,000 cells/well in 100 µL of DMEM/FBS in a 96-well plate and incubated at 37°C for five hours, then serum deprived for 1 hour. Different concentrations of insulin were added into the well and incubated for 30 minutes at 37°C, 5% CO_2 incubator. At the end of the incubation time, 100 µL of fatty acid mixture was added into the well, and kinetic readings were started immediately with a FlexStation Plate Reader. Traces **A** to **F** correspond to adipocytes with 160, 16, 8, 1.6, 0.16, and 0 nM insulin, respectively; traces **G** and **H** correspond to fibroblasts with 0 and 160 nM insulin, respectively.

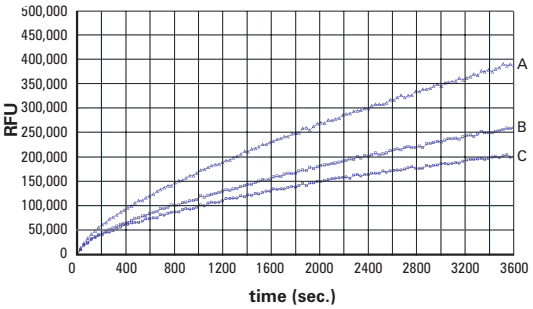


Figure 3. Leptin inhibitory effect. 3T3L1 adipocytes were plated at 50,000 cells/well/100 µL of DMEM/FBS in 96-well plate for five hours, then serum deprived for one hour with or without leptin (100 nM). Insulin at 10 nM was then added into the well and incubated for a further 30 minutes at 37°C, 5% CO_2 incubator. At the end of the incubation time, 100 µL of fatty acid was added into the well, and kinetic readings were started immediately with a FlexStation Plate Reader. Data courtesy of Cambridge Biotechnology. **A:** insulin, **B:** insulin and leptin, **C:** basal.

Optimized assay

The QBT™ Fatty Acid Uptake Assay is a single-step homogeneous fluorescent assay that provides real-time uptake kinetics and is ideally suited for high throughput screening applications in both 96-well and 384-well formats. The kit employs a BODIPY-dodecanoic acid fluorescent fatty acid analog coupled with Molecular Devices proprietary quench technology. The BODIPY label provides an ideal long chain fatty acid analog that behaves much like natural fatty acids: it becomes activated by acyl-CoA attachment, is incorporated into di-and triglycerides, and accumulates in intracellular lipid droplets. In addition, the BODIPY analog is a known substrate for fatty acid transporters since its uptake by adipocytes can be competed by non-labeled fatty acids. The elimination of radioactive compounds results in easier reagent handling, reduced disposal costs and eliminates safety risks associated with radiolabel assays.

Patented technology

QBT assay kits from Molecular Devices employ a quenching dye to reduce background fluorescence and improve the signal-to-noise ratio. The patented quench technology is offered to drug discovery and life science researchers exclusively by Molecular Devices, through the purchase of QBT Assay Kits.

Observe true biological activity

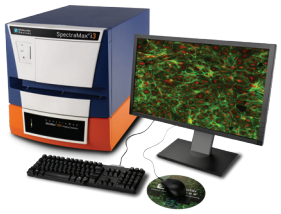
Consistent with the observation that FATPs are only expressed by differentiated cells, 3T3 L1 adipocytes take up the BODIPY analog and accumulate it in lipid droplets. In contrast, undifferentiated 3T3L1 fibroblasts do not transport or store the dye (Figure 1). Known activators and inhibitors of fatty acid uptake can be seen to stimulate or inhibit uptake in adipocytes in a dose-dependent manner, but show no effect on uptake in undifferentiated fibroblasts (Figures 2 and 3).

Rapid assay development

The streamlined homogeneous format makes the QBT Fatty Acid Uptake Assay Kit less labor intensive to run. With the single step mix-and-read procedure, the cells can be incubated with the reagents and are stable for several hours. Rapid analysis of the cells can be followed with the detection on any bottom-read fluorescence microplate reader from Molecular Devices.

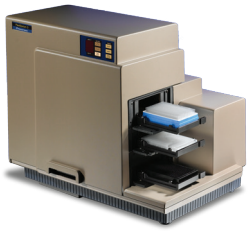
Ordering information		
Reagent	**Description**	**Part number**
QBT Fatty Acid Uptake Assay Kit (Explorer)	(10) 1-plate reagent vials* * Provides sufficient reagent for 10 plates (96- or 384-well)	R8132
QBT Fatty Acid Uptake Assay Kit (Bulk)	(10) 5-plate reagent vials* * Provides sufficient reagent for 50 plates (96- or 384-well)	R8133

Compatible with these Molecular Devices systems



SpectraMax® i3/i3x Multi-Mode Microplate Reader

SpectraMax® Paradigm® Multi-Mode Microplate Reader



FlexStation® 3 Multi-Mode Microplate Reader

SpectraMax® M Series Multi-Mode Microplate Readers

Gemini™ XPS Microplate Reader

FLIPR® Tetra High-Throughput Cellular Screening System

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