Molecular Devices

Product Insert

QBT™ Fatty Acid Uptake Assay Kit

Product # R8132 (Explorer Kit)
R8133 (Bulk Kit)

Introduction

About the Fatty Acid Uptake Assay Kit

The homogeneous QBT Fatty Acid Uptake Assay Kit from Molecular Devices Corporation provides a fast, simple and reliable fluorescence-based assay for the detection of fatty acid uptake in cells containing fatty acid transporters. The kit employs a BODIPY®-dodecanoic acid fluorescent fatty acid analog (BODIPY® 500/512 C1, C12[1]) coupled with Molecular Devices proprietary quench technology. With this kit, fatty acid uptake assays can be performed on any fluorescence microplate reader with a bottom-read mode in a no-wash procedure in which cells are incubated with the kit reagent and transferred to the plate reader for evaluation at the same time.

Existing detection methods either using radiolabeled fatty acids or fluorescent fatty acid analogs in fluorescence activated cell sorters (FACS) are very slow and not suitable for high throughput screening. Molecular Devices' homogeneous Fatty Acid Uptake Assay Kit offers such a benefit that the assay can be performed in 96- or 384-well microtiter plates in a simple mix-and-read procedure ideally suited for high throughput screening applications.

Problems associated with existing fatty acid uptake methods include:

- Disposal and personnel safety issues associated with radiolabeled assays
- Cell lysis and low temperature incubation steps complicate and prolong assay time
- Specialized instrumentation required (e.g. scintillation counters or cell sorters)

Molecular Devices has developed the QBT Fatty Acid Uptake Assay Kit, which not only eliminates the radioactivity, but also offers additional advantages over existing fluorescence-based assays.

Advantages of the QBT Fatty Acid Uptake Assay Kit include:

- Greatly simplified mix-and-read procedure with less hands-on time
- Fewer steps in the assay resulting in higher sample throughput
- Minimal perturbation of the cells maximizing cell competence
- Can be read on any bottom-read fluorescence microplate reader
- Can be used in either 96-well or 384-well format

Applications

The kit provides a homogeneous assay for fatty acid uptake. It is designed to work with murine or human adipocytes, or other cells known to contain fatty acid transport proteins (FATP).

Materials and Equipment

Kit Components

The following table lists the kit components.

Table 1: The QBT Fatty Acid Uptake Assay Kit (P/N R8132, R8133) contents.

Reagent	Description
R8132 (Explorer Kit)	10 vials sufficient for ten 96-well or 384-well plates. Each vial is sufficient for assaying one 96- or 384-well plate.
R8133 (Bulk Kit)	10 vials, sufficient for fifty 96-well or 384-well plates. Each vial is sufficient for assaying five 96 or 384-well plates.

Materials Required but not Provided

The following tables list the materials required but not supplied.

Table 2: Reagents and supplies

Reagent Item	Source
HBSS Buffer (1X Hank's Balanced Salt Solution with 20mM Hepes buffer and 0.2% fatty acid free BSA)	10X Hank's Balanced Salt Solution (#14065-056, Gibco or equivalent) 1M Hepes buffer solution (#9319, Irvine Scientific or equivalent) Fatty acid free BSA (A7511, Sigma) Water for cell culture (# 9312, Irvine Scientific or equivalent)

Storage and Handling

On receipt of the kit, store the Fatty Acid Uptake Assay Kit at –20°C. Under these conditions the reagents are stable for six months in the original packaging.

After formulation, the Loading Buffer is stable for up to eight hours at room temperature. Aliquots can be frozen and stored for up to 5 days without loss of activity.

Molecular Devices

QBT™ Fatty Acid Uptake Assay Kit Experimental Protocol

Cell Handling

The homogeneous Fatty Acid Uptake Assay Kit is designed to work in cells containing fatty acid transporters such as adipocytes. In this section we provide guidelines for how to set up the cells for use with the assay kit.

Differentiated 3T3-L1 adipocytes were prepared as described by Tengholm, A. et al (Sci STKE. 2003 Feb 11; 2003 (169):PL4). In short, 3T3-L1 fibroblasts were grown 2 days post-confluence in DMEM/FBS, and then for 2 days in DMEM/FBS supplemented with 0.83µM insulin, 0.25µM dexamethasone, and 0.25mM isobutylmethylxanthine. The medium was then changed to DMEM/FBS supplemented with 0.83 uM insulin for 2 days only and then maintained to DMEM/FBS alone for a further 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 initiation of differentiation.

- We recognize that a variety of cell handling conditions might be adopted at the discretion of the user based on standard operating procedures in the laboratory. Optimal cell conditions for the QBT Fatty Acid Uptake Assay require the creation of a confluent cell monolayer before placing the plates in the fluorescent microplate reader. In general, we recommend starting with 50,000-80,000 cells/well/100µl for a 96-well plate and 12,500-20,000 cells/well/25µl for a 384-well plate based on 3T3-L1 cells. It is recommended that you then centrifuge the plates at 1000 rpm for up to 4 minutes with **brake off**. It is also recommended that a triplicate of wells plated with growth medium ONLY be used as blank wells for data normalization.

- We find that adipocytes plated at the same day (4-5 hours, then serum deprived for 1 hour) give better results than that plated for overnight. For adherent cells, we recommend seeding cells overnight with a plating volume of 100 µl/well for 96-well plates or 25 µl/well for 384-well plates.

1. Preparation of 1X Loading Buffer

The following procedure is designed for one 96- or 384-well plate using cells prepared as described above.

1.1 To prepare the 1X HBSS + 0.2% BSA Buffer, add 2g of fatty acid free BSA, pipette 100ml of 10X Hank's Balanced Salt Solution and 20 ml of 1M Hepes buffer solution to 880ml water for cell culture.

1.2 Remove one vial of Component A and equilibrate to room temperature.

1.3 **Explorer Kit:** Dissolve contents of vial completely by adding **10ml** of 1X HBSS + 0.2% fatty acid free BSA Buffer. Mix by vortexing until the contents are completely dissolved.
Bulk Kit: Dissolve contents of vial completely by adding **10ml** of 1X HBSS + 0.2% fatty acid free BSA Buffer. Mix by vortexing until the contents are completely dissolved, then dilute the vial mixture in an additional **40ml** of 1X HBSS + 0.2% fatty acid free BSA Buffer for a total of **50ml**.

Warning: The components supplied are sufficient for proper cell loading. For optimum results it is important **NOT** to add any additional reagents or change volumes and concentrations.

2. Treatment of Cells with Test Compounds to Induce or inhibit Fatty Acid Uptake

2.1 Remove cell plates from the incubator, aspirate the medium from the wells, and deprive the cells with 90 µl/well (for 96 well-plates) or 20 µl /well (for 384 well-plates) serum free medium, incubate the cells at 37ºC, 5% CO_2 incubator for 1 hr. Add 10µl (for 96-well plates) or 5µl (for 384-well plates) of the test compounds or 1X HBSS (pH 7.4) as the compound diluent. For blank wells, the compound diluent is added.

2.2 Incubate at 37°C in a humidified CO_2 incubator for a desired period of time (30 minutes for 3T3-L1 cells treated with Insulin).

3. Loading the Cells with 1X Loading Buffer

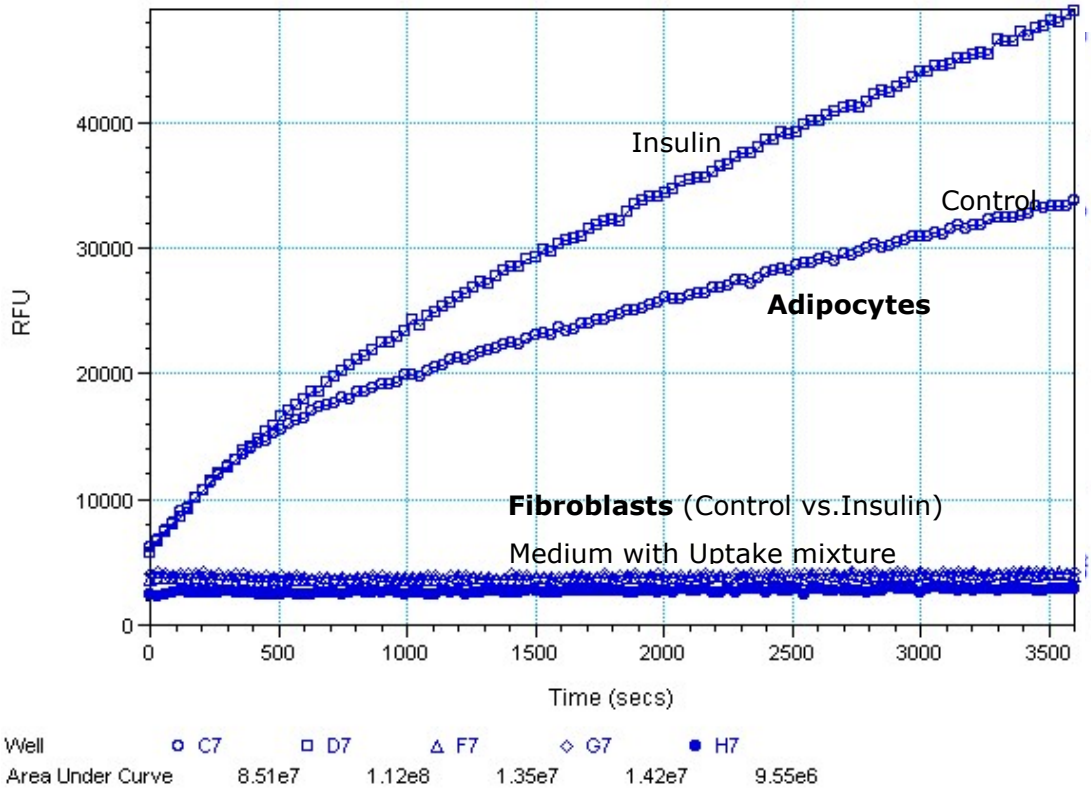
3.1 Remove cell plates from the incubator; add 100µl (for 96-well plates) or 25µl (for 384-well plates) per well of the 1X Loading Buffer (also for blank wells)

3.2 Transfer cell plates immediately to a fluorescence microplate reader for kinetic reading (every 20 seconds for 30-60 minutes) using a bottom-read mode.
Note: Measure with an excitation filter of 470-500 nm and an emission filter of 500-560 nm (maximum λ_{ex} = 485nm and λ_{em} = 515nm, cutoff 495 nm).

4. Data Analysis

The fluorescence in blank wells added with the growth medium is subtracted from the values for those wells with cells treated with the compound diluent or the test compounds. The background fluorescence of the blank wells can be varied depending on the sources of the microtiter plates or of the growth media.

Fatty Acid Uptake on 3T3-L1 Adipocytes vs. Fibroblast (+/- Insulin)



Molecular Devices

Product Use Limitations and Warranty

All Molecular Devices reagent products are sold For Research Use Only. Reagents may contain chemicals that are harmful. Due care should be exercised to prevent direct human contact with the reagent.

Each product is shipped with documentation stating specifications and other technical information. Molecular Devices products are warranted to meet or exceed the stated specifications. Molecular Devices' sole obligation and the customer's sole remedy are limited to replacement of the products free of charge in the event that the product fails to perform as warranted.

Molecular Devices Corporation makes no other warranties, either expressed or implied, including without limitation the implied warranties of merchantability and fitness for a particular purpose or use.

Molecular Devices Corporation is the exclusive licensee of a patented assay technology from Bayer A.G. (U.S. patent number 6,420,183, European patent number 0,906,572 and family members throughout the world). We are the only authorized provider of any assay incorporating enhancing, optimizing, quenching or masking agents as claimed in Bayer's patented technology. The purchase of this assay kit from Molecular Devices Corporation includes a non-exclusive right to practice Bayer A.G., U.S. Patent 6,420,183, European Patent 0,906,572, and foreign counterparts thereof, in conjunction with and only in conjunction with the use of this assay kit.

[1] This product is sold under license from Molecular Probes, Inc. for internal research and development use for monitoring fatty acid uptake by live cells, where internal research and development use expressly excludes the use of this product for providing medical, diagnostic, or any other testing, analysis or screening services, or providing clinical information or clinical analysis, in each case in return for compensation by an unrelated party, and internal research and development use also expressly excludes incorporation of this product into another product for commercialization, even if such other product would be commercialized for research and/or development use. Except as provided above, Molecular Probes, Inc. reserves all rights to the manufacture, use or sale of the fluorescent dye in this product under US Patent No. 4,774,339. BODIPY® is a trademark of Molecular Probes, Inc.

Molecular Devices

Molecular Devices Corporation
3860 North 1st Street
San Jose, CA 95134 USA
Email: info@moldev.com
www.moleculardevices.com

Sales Offices

USA 800-635-5577 • **UK**+44-118-944-8000 • **Germany** +49-89-9620-2340 • **Japan** +06-6399-8211
Check our web site for a current listing of our worldwide distributors.

R3519 Rev. E 03/02/17