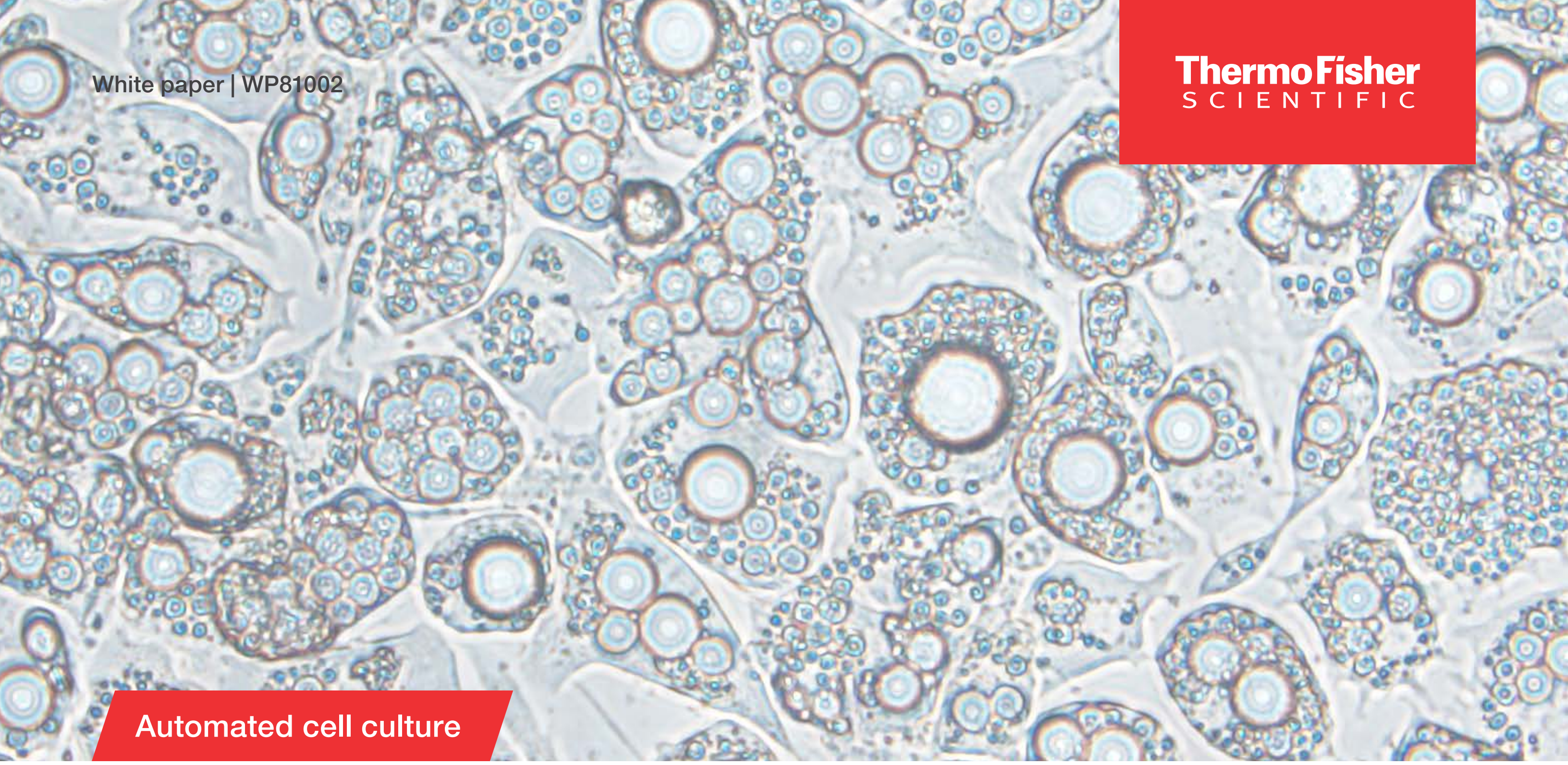

Automated cell culture

# Cell culture maintenance and differentiation on the Tecan Fluent Automation Workstation

## Automated seeding, medium exchange, and incubation of 3T3-L1 cells using the Tecan Fluent Automation Workstation and Cytomat automated incubator

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**Keywords**

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### Introduction

Over the last couple of decades, 3T3-L1 cells have played an important role in advancing research for the treatment of metabolic diseases like diabetes and obesity. These cells, under optimal conditions, can differentiate from a fibroblast-like morphology into adipocyte-like cells that accumulate lipids in structures known as lipid droplets.

This white paper describes the successful automated maintenance and differentiation of 3T3-L1 cells into adipocyte-like cells through medium exchange using the Tecan® Fluent® 780 Automation Workstation and incubation of preadipocyte cells under environmentally stable and sterile conditions over a period of three weeks using an integrated Thermo Scientific™ Cytomat™ 2 C450-LiN Automated Incubator.

### Experimental

#### Automation workstation

Experiments were conducted on a Fluent 780 system, which includes an eight-channel Air Flexible Channel Arm (Air FCA), a Multiple Channel Arm (MCA) with an extended volume adapter for pipetting up to 500 µL with 96 tips in parallel, and a long Robotic Gripper Arm (RGA) to reach below the Dynamic Deck. A Cytomat 2 C450-LiN Automated Incubator with two stackers—for 21 and 10 microplates, respectively—was integrated with the Fluent 780 system, enabling the storage and incubation of cells in microplates throughout the entire preadipocyte differentiation period (Figure 1). The incubator's parameters were set at 37 °C and 5% $CO_2$ to provide an ideal and stable environment for cells to grow and differentiate into adipocytes. The Cytomat 2 C450-LiN also offers the ContraCon™ decontamination routine for an automated decontamination cycle, simplifying cleans and eliminating variability in disinfection without harmful gases.

**Figure 1. The Fluent Automation Workstation and integrated Cytomat 2 C450-LiN Automated Incubator used during the cell differentiation experiment.**

To ensure a clean environment for sterile cell culture applications, a vertical laminar flow HEPA hood with UV light (Bigneat) was integrated with the Fluent system. Liquid handling tasks were conducted with the Air FCA and MCA, using 1,000 µL and 150 µL filtered disposable tips. Sterile 50 mL Falcon™ tubes were placed into a tube runner for pipetting into sterile 96-well microplates (Greiner Bio-One Part No. 655090) with the Air FCA for the cell seeding step, whereas sterile 300 mL troughs (INTEGRA Biosciences) were used with the MCA for the medium exchange steps. Individual scripts were developed using the Tecan® FluentControl® software for cell seeding, medium exchange with the Air FCA and MCA, and incubation in the Cytomat 2 C450-LiN Automated Incubator.

## Cell thawing and expansion

3T3-L1 cells (Zen-Bio) were thawed at passage 8 following the manufacturer's protocol. Cells were then seeded in T175 flasks at 450,000 cells per flask and cultured in the 3T3-L1 preadipocyte medium (Zen-Bio Part No. PM-1-L1). After a brief amplification period, cells were manually harvested with 0.05% trypsin and 0.02% EDTA (PAN-Biotech Part No. P10-023500) once they reached around 70% confluence, until passage 11.

## Cell seeding, medium exchange, and plate incubation

3T3-L1 cells were seeded in six black 96-well microplates (Greiner Bio-One Part No. 655090) at 3,000 cells per well in a 150 µL volume. All outer wells were filled with phosphate-buffered saline (PBS), so only 60 wells were used for the actual plating. Cells were then maintained in the automated incubator at 37 °C and 5% $CO_2$.

Three microplates were placed in each of the two stackers—at the top, middle, or bottom—to assess consistent cell growth and differentiation and confirm that environmental conditions were stable regardless of the plate location inside the incubator. Plates 1–3 were located in the 21-position stacker on the right of the incubator, at the bottom, middle, and top, respectively. Plates 4–6 were placed in the 10-position stacker on the left of the incubator, at the bottom, middle, and top, respectively.

The 3T3-L1 preadipocyte medium was exchanged using the MCA, allowing cells to continue growing until they reached 100% confluence. 18 boxes of 150 µL MCA 96 filtered tips were used per medium exchange step, following Zen-Bio's recommended feeding volumes for each step of the differentiation protocol. Once the cells were confluent (three days after seeding), an additional incubation of 48 hours was conducted before initiating the differentiation step. Five days post-seeding (day 0), the culture medium was changed to the differentiation medium (Zen-Bio Part No. DM-2-L1) as shown in Figure 2, and cells were cultured for three days. On day 3, the differentiation medium was removed and replaced with adipocyte maintenance medium (Zen-Bio Part No. AM-1-L1). Cells were incubated up to day 14 with regular adipocyte maintenance medium exchange every two to three days.

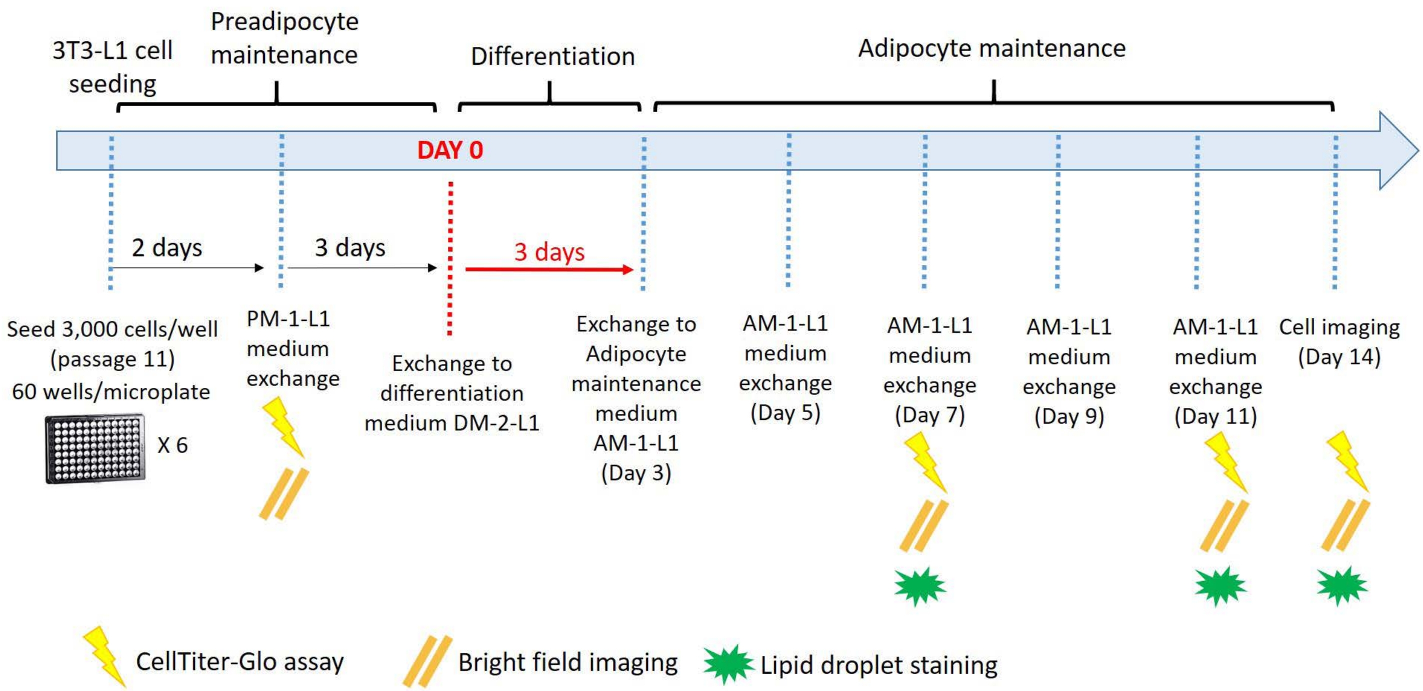


**Figure 2. 3T3-L1 cell differentiation protocol.**

## Cell viability assay

To ensure cells were viable and in good health, a cell viability assay was conducted on day 2 post-seeding, as well as days 7, 11 and 14 post-differentiation. Four to six wells per microplate were used to assess cellular health with Promega's CellTiter-Glo™ assay, following the manufacturer's protocol. The Air FCA was used to dispense the CellTiter-Glo reagent and execute the mixing step. All six assay plates were incubated at room temperature for 10 minutes before measuring the luminescent signal on the Spark™ Multimode Microplate Reader with an integration time of 1 second per well.

## Lipid droplet staining and cell imaging

Intracellular lipid droplets are cytoplasmic organelles that accumulate in fat cells and are mainly involved in the storage and regulation of triglycerides and cholesterol esters. The lipid droplets in live 3T3-L1 adipocytes were stained manually under sterile conditions using LipidSpot™ 488 Lipid Droplet Stain, 1,000X in DMSO (Biotium Part No. 70065-T) with bright green fluorescence. LipidSpot was diluted in adipocyte maintenance medium to reach 1X final concentration after being added to the cells, which were then incubated at 37 °C in the dark for 30 minutes in a non-automated incubator. An Olympus™ IX81 automated microscope was used for bright field and green fluorescence imaging of the cells on days 7, 11 and 14. Both 20x and 40x magnifications were used in order to properly visualize the formation of lipid droplets, and their increase in size and number.

## Results and discussion

The homogenous cell assay using CellTiter-Glo assay on the Spark multimode reader determined the number of viable cells that were metabolically active in the wells, based on the quantitation of adenosine triphosphate (ATP, Figure 3). Data from the first cell viability analysis, conducted 48 hours after cell seeding, showed a difference from the rest of the time points due to the cells continuously dividing in the growth phase at this stage. Once cells reached 100% confluence and the differentiation process started, similar luminescence results can be observed on days 7 and 11. Toward the end of the differentiation protocol, cell viability appeared to have decreased as expected, considering these adipocyte-like cells are optimally used for testing between day 11 and day 14 post-differentiation. More importantly, results showed consistency across plates for each day of the measurements, which indicates that the environmental conditions inside the automated incubator were stable and not dependent on the plate location. Due to technical issues, the measurement for plate 4 on day 14 post-differentiation could not be captured.

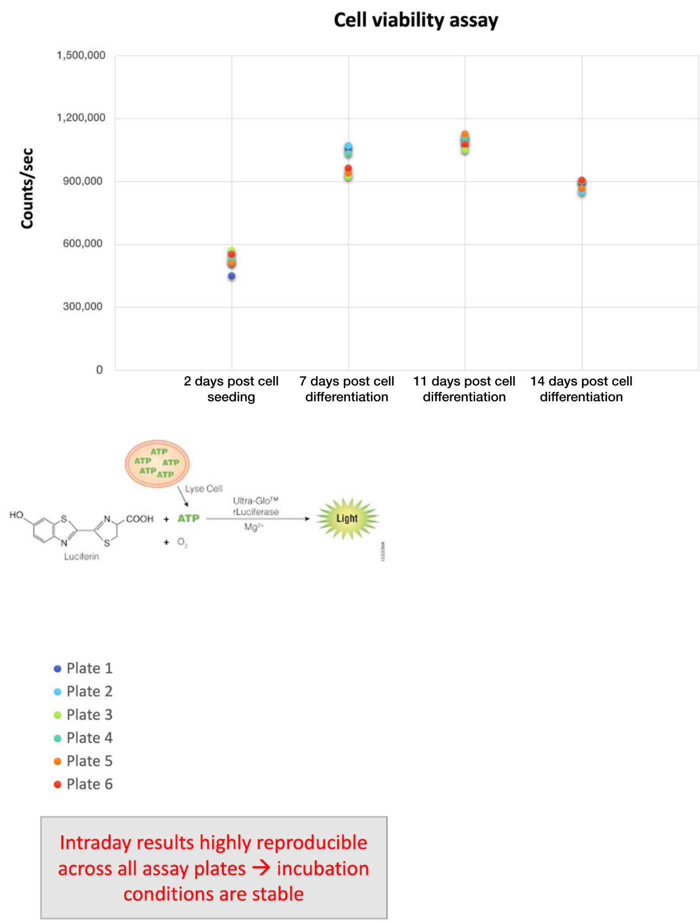


**Figure 3. CellTiter-Glo viability assay on different days across the 3T3-L1 cell differentiation protocol.**

Cells were imaged using bright field and fluorescence imaging (green channel) modes at multiple time points throughout the protocol, as shown in Figure 4. Both the bright field images (Figure 4A) and the fluorescence images (Figure 4B) clearly show the accumulation of lipid droplets and their increase in size and number, indicating successful differentiation of 3T3-L1 preadipocytes into adipocyte-like cells. Using the LipidSpot staining technique is advantageous, as the cells can be imaged only 30 minutes after addition of the dye, and the protocol does not require any washing steps, with minimal background staining of other cellular organelles.

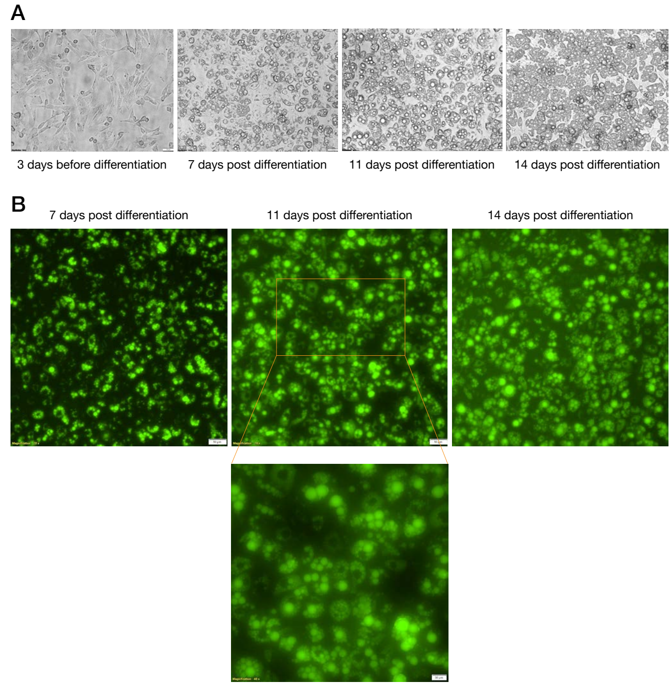


**Figure 4. Images of the differentiation of 3T3-L1 preadipocyte cells into adipocyte-like cells.** (A) Bright field images of cultured 3T3-L1 cells taken 48 hours after cell seeding and on days 7, 11 and 14 post-differentiation, using 20x magnification. (B) Fluorescence imaging using the green channel, conducted on days 7, 11 and 14 post-differentiation, using 20x and 40x magnifications to better visualize the lipid droplets accumulating over time.

## Conclusions

The data presented here shows that the Fluent 780 Automation Workstation is well suited to conduct this type of long-term cell culture maintenance and achieve reproducible results. The Cytomat 2 C450-LiN Automated Incubator provides the ideal solution for mid-capacity incubation and storage of culture plates, enabling stable cell culture conditions and fast access of the microplates in a safe and consistent environment. No contamination issues were observed during the experiment, demonstrating that the system helps ensure a clean and sterile environment for cell culture maintenance applications.

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