Real-Time Experimental Control (REC™) for live cell fluorescence reporter applications.

Application Note

AUTOMATED MONITORING OF THE REPORTER ACTIVITY OF HFOB1.19 CELLS FOLLOWING INDUCTION OF OSTEOGENIC DIFFERENTIATION BY TEMPERATURE SHIFT

INTRODUCTION

Performing cell-based assays with fluorescent reporters can be a laborious process. Induction of the cells, then monitoring using common fluorescence microscopes, multimode readers or flow cytometers requires a high degree of manual interaction with both the microplates and the detection instrument. Multimode reader approaches offer higher throughput compared to other systems, but do not provide any information about the signal distribution across the whole cell population. Flow cytometers and microscopes deliver cellular information and enable signal distribution analysis, but the overall throughput is very limited.

Tecan's Spark® Cyto is the first live cell plate reader with real-time imaging cytometry, enabling semi-automated workflows for cell culture experiments. Combining multimode detection and fluorescence imaging allows visual and functional examination of cell samples within a single experiment.

The Spark Cyto is equipped with a high-end imaging module incorporating three user-selectable objectives, four fluorescence channels, LED-based illumination and a highly reliable and rapid autofocusing system. Operated via the user-friendly and intuitive SparkControl™ software, Spark Cyto enables automation of long-term kinetic assays, with advanced features – such as full environmental control, kinetic control software and kinetic conditioning capabilities – to provide a walkaway solution for complex experimental set-ups.

Many live cell kinetic experiments require specific actions at different points in the experiment, for example, the addition of a compound once a certain confluence is reached. Spark Cyto's Real-Time Experimental Control (REC) combines advanced kinetic conditioning features and a unique real-time analysis function to allow certain actions – such as reagent injection, temperature increase/ decrease or gas concentration changes – to be triggered automatically within a kinetic experimental workflow.

This Application Note shows how Spark Cyto can be used to monitor the expression of enhanced green fluorescent protein (eGFP) in human fetal osteoblast (hFOB1.19) cells. eGFP expression was controlled by the osteocalcin promoter, and was used as a reporter for osteogenic differentiation caused by a temperature shift. REC features were included in the software protocol, allowing the temperature shift to be carried out automatically once a defined confluence threshold was reached.

MATERIALS AND METHODS

Adherent hFOB1.19 eGFP cells were harvested from 80-90 % confluent cultures, resuspended in fresh complete medium (DMEM + supplements) and counted using trypan blue staining. Cells were seeded at a concentration of $5x10^3$ cells/well or $2.5x10^3$ cells/well in DMEM-F12 +10 % FBS or MEM +20 % FBS in a Tecan 96-well microplate. Four replicate were generated for each condition. Cells were incubated inside the Spark Cyto, using the Humidity Cassette, at 34 °C and 5 % CO_2 (see Table 1). Confluence was measured every four hours throughout the analysis period (183 h) and, when the culture reached a confluence of >80 %, the temperature was increased to 39 °C to induce differentiation using the unique kinetic conditioning function in the system's SparkControl software. Combining kinetic conditioning with the software's 'user intervention' feature offered a semi-automated workflow that allowed manual media changes and refilling of the Humidity Cassette's reservoir (every 48 h) to ensure the best possible cell growth. Detection of eGFP expression following induction at 39 °C was also completely automated.

Parameter	Setting
Temperature control	On
Target temperature	34 °C
CO_2 control	On
Target conc.	5 %
Plate	TEC96ft_cell
Mode	Kinetic
Kinetic cycles	41
Interval time (hh:mm:ss)	04:00:00
Measurement mode	Fluorescence imaging
Application	User defined
Channels	Bright field, Green
Objective	4x
Pattern	User defined
Bright field channel	
Focus offset	-15 µm
Sensitivity	50 %
Object size	8-30 µm width, 8-30 µm length
Fill holes, with size <x µm^2	0 µm
Green channel	
LED intensity	100 %
Focus offset	0 µm
Exposure time	300 ms
Sensitivity	50 %
Object size	8-30 µm width, 8-30 µm length
Analysis type	Segmentation
Real-time control	
Mode	Condition
Type	Start at value
Executed once	
Input data	Label 1 Confluence
Reference	A2
Operation	>80 %
Mode	Condition
Type	Start at cycle
Operation	At cycle, 10, 20, 30, 40, 50
User intervention	Check humidity and medium change

Table 1: Software protocol to assess reporter activity on the induction of osteogenic differentiation by temperature shift.

RESULTS

hFOB1.19 cells cultured inside the Spark Cyto reader showed a steady and homogeneous growth over the plate (CV = ≤10 %) from 20 to 80 % confluence. Cell growth continued, showing identical dynamics, following an automated temperature shift from 34 to 39 °C at 80 % confluence, finally reaching 99 % confluence after 183 hours (see Figure 1). The temperature shift led to the expression of eGFP in the cells, increasing the green fluorescent signal significantly in all samples (see Figure 2).

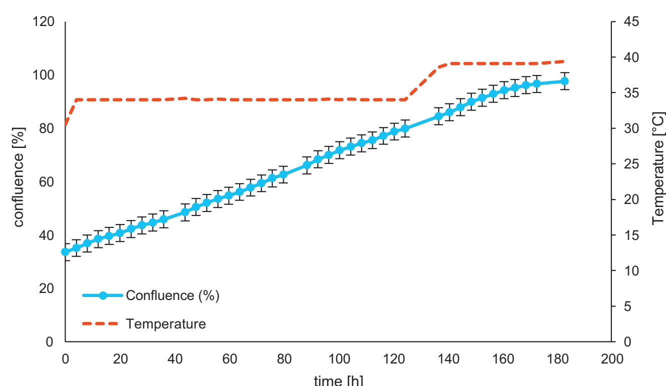

Figure 1: Growth of hFOB1.19 cells with a kinetic condition at 80 % confluence triggering a temperature increase to 39 °C.

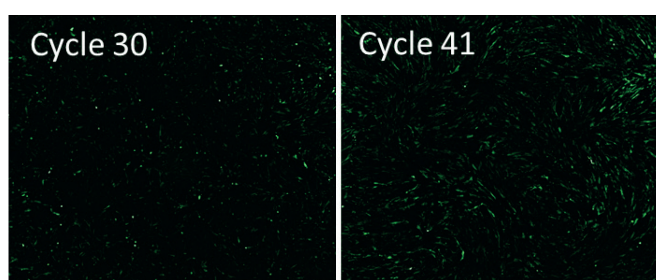

Figure 2: Induction of eGFP expression in hFOB cells by temperature shift from 34 °C (cycle 30) to 39 °C (cycle 41).

CONCLUSIONS

The results acquired in this study demonstrate the capability of the Spark Cyto to conduct long-term live cell assays, such as live cell fluorescence reporter applications, for up to seven days. This is due to the full set of integrated environmental control features, including temperature regulation, gas control and evaporation protection. Special software features, such as kinetic conditioning, help to minimize hands-on time by automating critical steps of the workflow, for example the induction of temperature shifts at a certain confluence level.

ABBREVIATIONS

DMEM	Dulbecco's modified Eagle's medium
eGFP	enhanced green fluorescent protein
FBS	fetal bovine serum
hFOB1.19	human fetal osteoblast 1.19
LED	light-emitting diodes
MEM	modified Eagle's medium
REC	Real-Time Experimental Control

About the author
Dr. Christian Oberdanner is Senior Application Specialist at Tecan Austria. He studied molecular biology at the University of Salzburg with a strong focus on cell- and tumor biology. Christian started to work for Tecan Austria as external scientific consultant in 2005 and permanently joined the company in 2006. Since then he held several roles as Application Scientist, Application Specialist and Product Manager in research and development as well as in the sales and marketing department. Christian's priority within Tecan are multimode microplate reader applications and cell imaging.

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Tecan has not independently validated this method with all possible sample types or analytical uses and is providing this example method as a convenience to users. The user must independently evaluate and validate: (a) the suitability of the method for their use, (b) their ability to process samples of their choosing following the method; and (c) their ability to proficiently perform the method in their facility with their personnel.

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