APPLICATION NOTE 90549

Investigation of cell proliferation of HEK239 and HepG2 cells in the Cytomat 10 C Incubator

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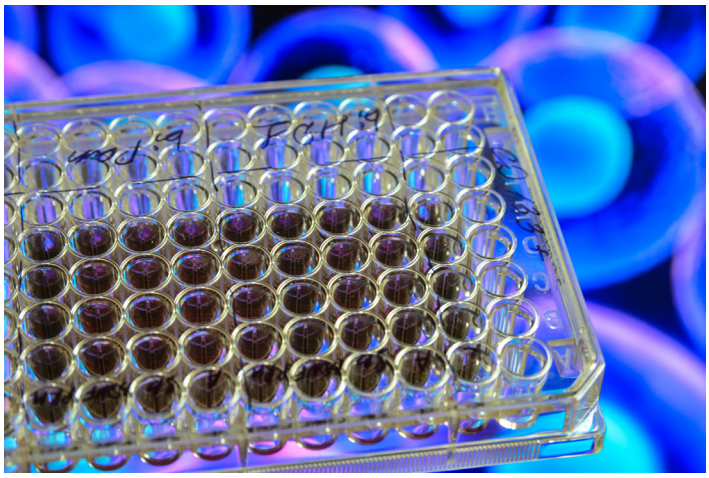

Goal

To investigate the proliferation of cells within the Thermo Scientific™ Cytomat™ 10 C automated incubator.

Introduction

The use of automated systems has become standard for research and drug discovery processes in the pharmaceutical industry. Process tasks such as robotic handling of vessels, incubation of cells, and analytical measurements, are covered by specific technical equipment with corresponding software control. To achieve reliable results, robotic systems need to be tested with real biological samples. During a cell culture process, incubation is one of the most critical and time-consuming steps and in many cases is underestimated regarding reliability and performance.

In this study we investigated cell proliferation of HEK293 and HepG2 cells in the Cytomat 10 C incubator. HEK293 (human embryonic kidney cell line) and HepG2 (human liver cancer cell line) cells are both commonly used cell types for pharmaceutical applications. HEK293 are used for a variety of basic cell biology assays as well as an expression system for recombinant proteins. HepG2 function as an *in vitro* system for human liver metabolism or drug targeting studies.

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Equipment

- Cytomat 10 C automated incubator
- Thermo Scientific™ Heracell™ CO_2 incubator
- Cytation™ 5 cell imaging reader, BioTek®
- GloMax® Discover Microplate Reader, Promega™

Reagents and standards

Promega RealTime-Glo™ MT Cell Viability Assay Kit

Samples

HEK293 and HepG2 cells

Procedure

HEK293 and HepG2 cells were seeded in 96-well and 384-well plates, each incubated on different positions within the Cytomat 10 incubator (37 °C / 5% CO_2 / humidified atmosphere).

Table 1. Seeding density of cells in 96-well and 384-well plates

Cell type	96-well plate	384-well plate
HEK293	10.000 cells / well	5.000 cells / well
HepG2	10.000 cells / well	2.500 cells / well

The Promega RealTime-Glo™ MT Cell Viability Assay Kit was used to monitor the viable cells during the culture time of 72 h. Cell viability was assessed every 24 h via luminescence measurement with the GloMax® Discover Microplate Reader. In addition, automated microscopic imaging was carried out using the BioTek Cytation™ 5 cell imaging reader. A control group with 2 plates per cell type and plate format was incubated in a standard Heracell CO_2 incubator.

Figure 1. Procedure to assess viability and morphology of the cells. Seeding of the cells in 96-well and 384-well plates, incubation and analyzing steps during 72 hours.*

Results

HEK293 cells

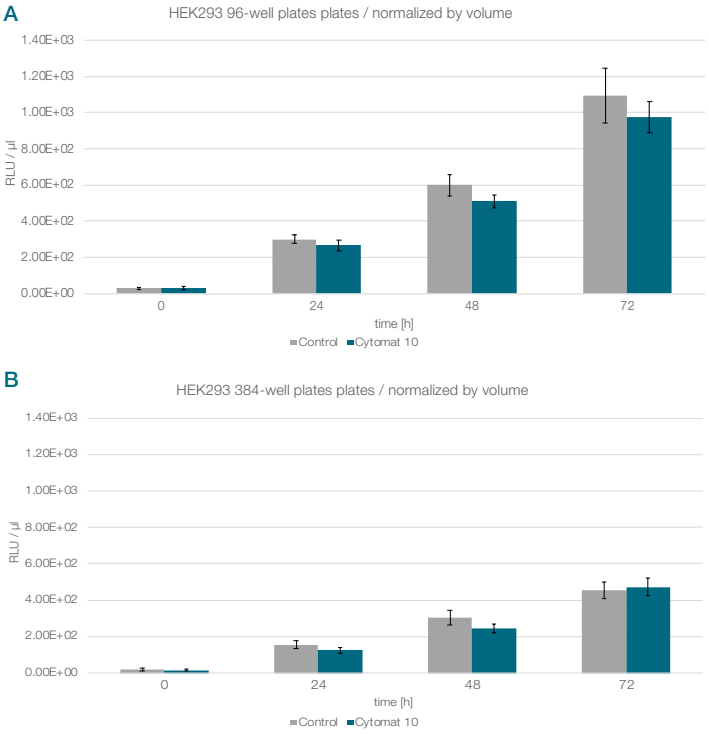

Figure 2. Luminescence measurement of HEK293 cells. Relative luminescence units per µL over time for control plates and plates that were processed in the Cytomat 10. A) 96-well plates; B) 384-well plates.

HepG23 cells

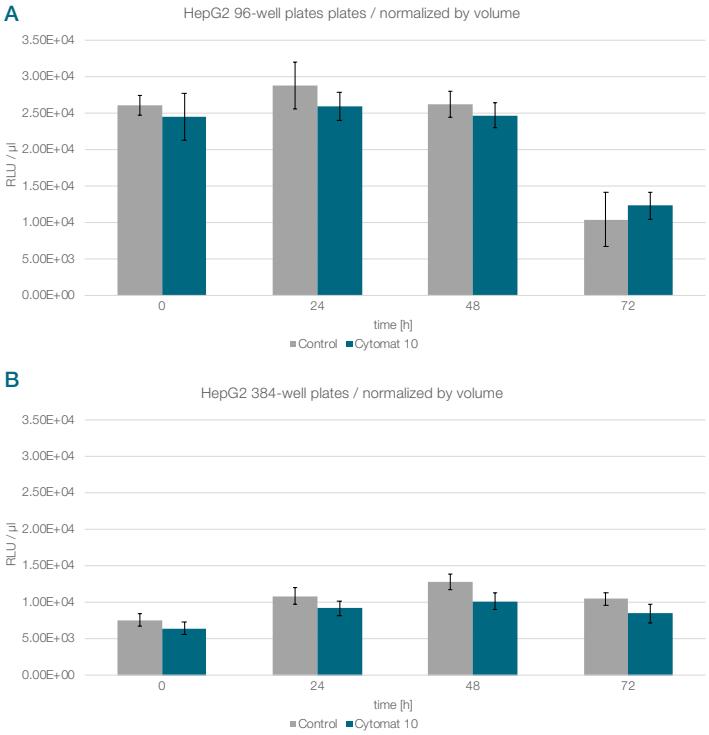

Figure 4. Luminescence measurement of HepG2 cells. Relative luminescence units per µL over time for control plates and plates that were processed in the Cytomat 10. A) 96-well plates; B) 384-well plates.

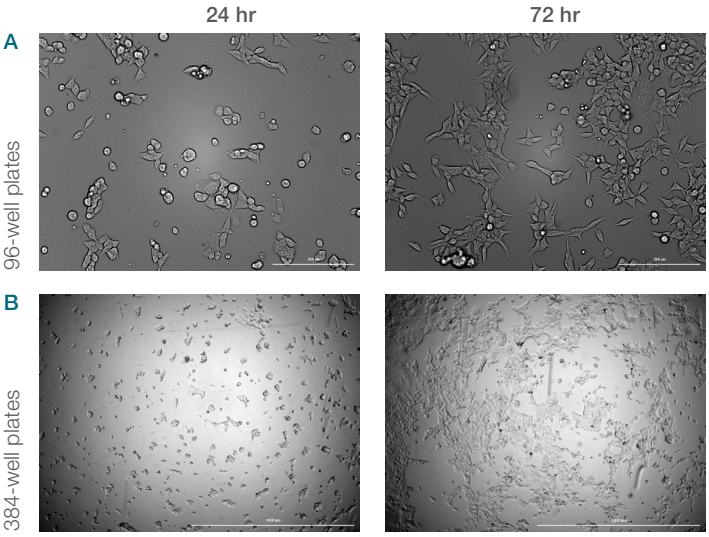

Figure 3. Microscopic images of HEK293 cells. Images of HEK293 cells 24 hr and 72 hr after seeding, incubated in the Cytomat 10. A) 96-well plates; B) 384-well plates.

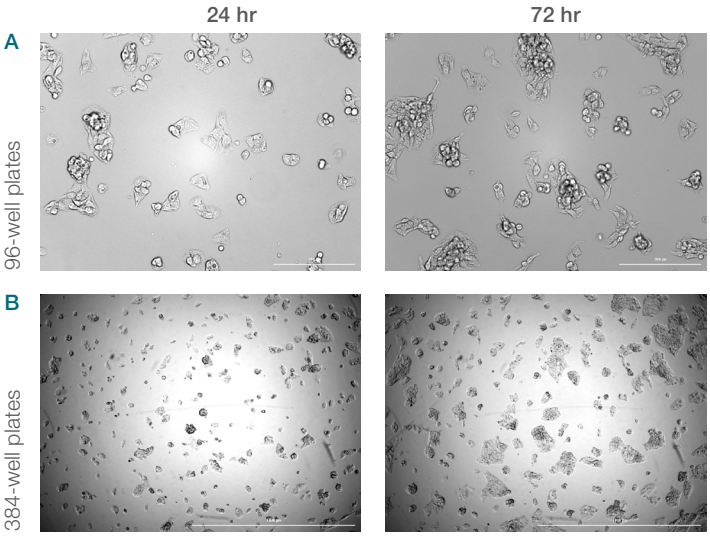

Figure 5. Microscopic images of HepG2 cells. Images of HepG2 cells 24 hr and 72 hr after seeding, incubated in the Cytomat 10. A) 96-well plates; B) 384-well plates.

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Conclusion

Luminescence measurements indicated a cell specific proliferation during the incubation in the Cytomat 10 C incubator, which was comparable to the control group in the Heracell CO_2 incubator. Cell culture conditions on all tested positions in the incubator were comparable and did not affect cell proliferation rates. Furthermore, no negative effects of the robotic handling steps of plate loading and unloading for daily luminescence measurement were detected. Microscopic images further proved that the HEK293 as well as HepG2 cells were proliferating during the culture time in both cell culture formats within the incubator. During the experiments no edge effects caused by evaporation could be observed (relative humidity >90%).

This study demonstrated the successful use of the Cytomat 10 C incubator for two pharmaceutical relevant cell lines. The integration of the incubator into robotic platforms provides a robust and reliable system for automated cell culture processes.

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