Application Note

CL-Quant Add-on Module

A method for evaluating the cell growth of a human hepatoma cell line, HepG2 cells using "Cell Confluency"

< BioStation CT usage example >

Benefits

- Using the CL-Quant image analysis software with the CL-Quant Add-on Module "Cell Confluency" makes it possible to measure cell confluency more objectively and quantitatively than the visual inspection that has been commonly used. This helps to standardize decision criteria for good cell culture procedure.
- A HepG2 cell line is commonly used as a human hepatic cell model for evaluation of hepatic drug metabolism and hepatotoxicity. To obtain reproducible test results using HepG2 cells, it is important to use good cultured cells at proper timing. To grow good cells, it is important to perform cell passaging when it is determined that cell growth has reached the appropriate level of cell confluency. The Add-on Module "Cell Confluency" supports determining the proper timing for passaging cells.

Observation device

- BioStation CT (Nikon, MLA10000)

Software

- CL-Quant ver. 5.02 (Nikon, MLS21000)

CL-Quant Add-on Module

- MA-PC-UR-AR01 Cell Confluency (Nikon, MLS30201)

Cells

- Human hepatoma cell line, HepG2 (JCRB Cell Bank, JCRB1054)

Materials

- DMEM, low glucose, pyruvate with L-Glutamine without HEPES (Thermo Fisher Scientific, 11885084)
- Fetal Bovine Serum (FBS), certified, United States (Thermo Fisher Scientific, 16000044)
- PBS(-), pH 7.4 (Thermo Fisher Scientific, 10010023)
- TrypLE™ Select Enzyme (1x), no Phenol Red (Thermo Fisher Scientific, 12563011)
- Costar® 6-well Clear TC-treated Multiple Well Plates (Corning, 3516)

Methods

HepG2 cells were inoculated with TrypLE™ select, and were plated into a 6-well plate at the following cell density 0.5×10^5, 0.75×10^5, 1.0×10^5 cells/well in DMEM supplemented with 10% FBS. The cells were cultured in a BioStation CT at 37˚C in a humidified atmosphere of 5% CO_2.

Phase contrast images of 8×8 fields (approx. 16.0 mm×16.0 mm) at the center of a well were automatically captured for 3 days, with a 10× objective lens every 3 hours from 2 hours after cell seeding confirmed that the cells attached. Auto-focusing was adjusted on the first field of view for a well and then images were automatically taken using that auto-focusing setting within that well.

The obtained image data were analyzed using the Add-on Module "Cell Confluency" to automatically measure the cell region to image size ratio. The masked images and the measured value of the area where cells are localized were confirmed on the operation screen and output in Microsoft Excel® format. The output values were converted to a cell confluency ratio (%), and graphed over time.

Results

The image analysis using the Add-on Module "Cell Confluency" demonstrated that when HepG2 cells were seeded at a density of 1.0×10^5 cells/well, the cell confluency (%) reached approximately 30, 50 and 70% after 14, 32 and 50 hours respectively (Fig. 1).

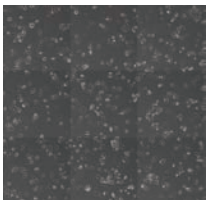
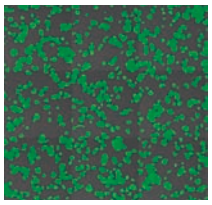
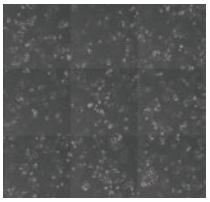
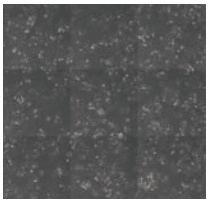
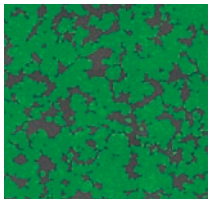
Culture time (hours)	Cell occupancy rate (%)	Phase contrast image	Masked image
14	31.0		
32	47.9		
50	70.3		

Figure 1. Phase contrast images and analyzed images.
Representative phase contrast and masked images of a 3×3 field of view were shown when HepG2 cells were seeded at a density of 1.0×10^5 cells/well. The obtained values of the cell confluency ratios and culture times were indicated.

Then we graphed the cell confluency over time during the entire culture period for the wells seeded at various cell densities (Fig. 2). The graph shows that confluency curves were different at different seeding cell densities.

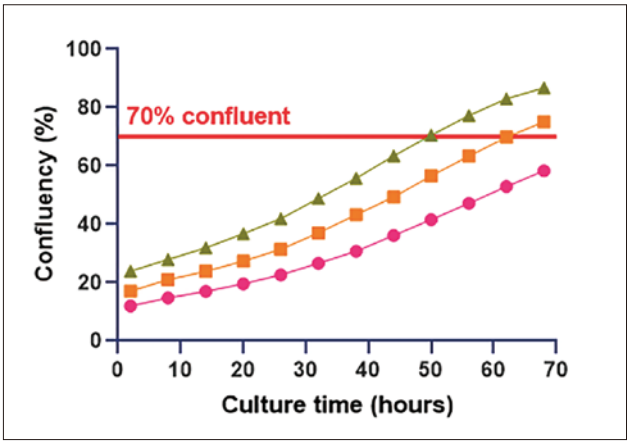


Figure 2. Time course of cell confluency at different seeding cell densities
HepG2 cells were seeded at the cell density of 5×10^4 (magenta), 7.5×10^4 (orange), and 1.0×10^5 (green) cells/well and observed every 3 hours for 3 days. The numeric values of the cell confluency obtained by CL-Quant were converted to a percentage and a line was added to confirm the time to reach 70% confluence.

Summary

- Using the CL-Quant image analysis software with the Add-on Module "Cell Confluency" makes it possible to measure the cell confluency automatically from phase contrast images during the HepG2 cell culturing process.
- By referring to the masked image on the operation screen of CL-Quant, it is easily confirmed that the cell occupied area is recognized correctly.
- Monitoring the condition of cells during the culturing process and obtaining data on cell confluency can help determine the timing for assays and cell passaging.
- The obtained numeric measured values can be output in Microsoft Excel® format.

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