Application Note

Cell Analysis 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 NIS-Elements image analysis software with the Cell Analysis 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 Cell Analysis Module "Cell Confluency" supports determining the proper timing for passaging cells.

Observation device

- BioStation CT (Nikon, MLA10000)

Image Analysis Software

- NIS-Elements AR ver. 5.30.02 (Nikon, MQS31000)
- NIS-A General Analysis (Nikon, MQS43110)
- NIS-A Upgrade to GA3 (Nikon, MQS43150)

Image Conversion Software

- ND2 Generator for BS-CT (Nikon)

Cell Analysis Module

- PC-AR-01 Cell Confluency (Nikon, MQS60001)

Cells

- Human hepatoma cell line, HepG2 (JCRB Cell Bank, JCRB1054)(1)

Materials

- DMEM, low glucose, pyruvate (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 dissociated using PBS and TrypLE™ Select, and were plated into a 6-well plate at the following cell density 0.5×10^6, 0.75×10^6, 1.0×10^6 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. 6.4 mm × 6.4 mm) at the center of a well were automatically captured for 3 days, with a 10× objective lens every 6 hours from 2 hours after cell seeding confirmed that the cells attached. Autofocusing 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 converted to the ND2 format image data using the ND2 Generator for BS-CT, and then analyzed using the NIS-Elements and Cell Analysis Module "Cell Confluency" to automatically measure the cell confluency (%). The masked images and the measured value of the region where cells are attached were confirmed on the operation screen. Output values in CSV format were graphed to confirm the times when the cell confluency reached approximately 30, 50 and 70%.

Results

When HepG2 cells were seeded at a density of 1.0×10^6 cells/well and observed every 6 hours from 2 hours after cell seeding, the cell confluency (%) reached 30, 50 and 70% after approximately 14, 32 and 50 hours respectively (Fig. 1).

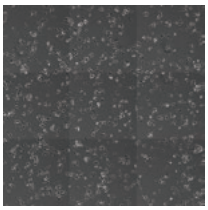
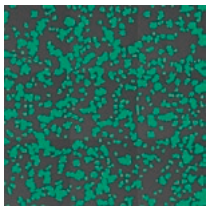
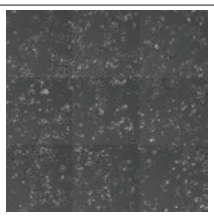
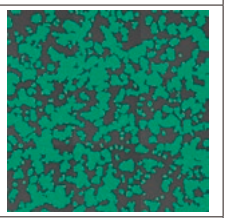
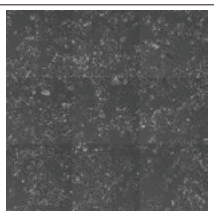
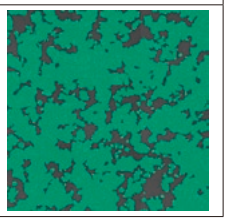
Culture time (hours)	Cell confluency (%)	Phase contrast image	Masked image
14	31.1		
32	48.5		
50	71.1		

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 1.0×10^6 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.

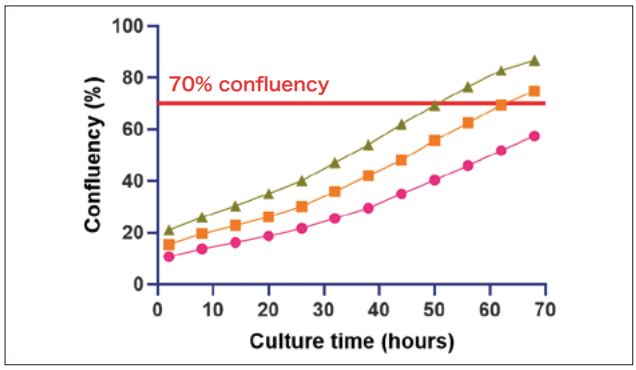


Figure 2: Time course of cell confluency at different seeding cell densities

HepG2 cells were seeded at the cell density of 0.5×10^6 (magenta), 0.75×10^6 (orange), and 1.0×10^6 (green) cells/well and cell confluency of each well was indicated over time. A line was added to confirm the time taken to reach 70% confluence.

Summary

- Using the NIS-Elements image analysis software with the Cell Analysis 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 NIS-Elements, 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 CSV format and graphed using software such as TIBCO Spotfire® and Microsoft Excel®.

Reference

1. Taisuke Mori et al., Combination of hTERT and bmi-1, E6, or E7 induces prolongation of the life span of bone marrow stromal cells from an elderly donor without affecting their neurogenic potential, *Mol Cell Biol.*, 25(12): 5183-5195, 2005.

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