

Reproducible dispensing of live cells with the Multidrop Combi Reagent Dispenser

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Introduction

High throughput cell-based assays are an important part of drug screening, and successfully dispensing viable cells is a key step in the process. Automated reagent dispensers are used to achieve higher throughput in screening laboratories. Dispense must not only be quick, it must also be gentle enough to ensure cell viability.

Here we present the process of dispensing live HeLa S3 cells with the Thermo Scientific™ Multidrop™ Combi Reagent Dispenser into 96- and 384-well plates and compare the viability of cells to conventional hand-pipetting. The peristaltic pump technology used in the Multidrop Combi system and optimised dispensing cassettes assure fast dispensing of cells on any multi-well plate and well size for any cell-based assay.

Materials and methods

Culturing the cells

The HeLa S3 cells were grown in adherent culture, Kaighn's media with FBS and penicillin/streptomycin, in cell culture flasks at 37°C with 5% CO₂. The cells were maintained at 0.2 - 0.6 x 10⁶ cells/ml by passaging them 1:5 - 1:8 every three days.

Detaching and dispensing

The number of cells was calculated after the cells were detached from the bottom of the flask with trypsin and



the media was changed. The Multidrop Combi Reagent Dispenser was used to dispense the cell suspension and reagents. The tubings were pre-wetted with PBS and primed with cell suspension before dispensing the actual samples. When dispensing several plates, the cassette was primed before each plate. The Thermo Scientific™ Finnpipette™ Focus pipette was used to dispense the cells for the control wells.

The Multidrop Combi system was used to dispense 100 µl volumes of the cell suspension into wells of Thermo Scientific™ Nunc™ F96 MicroWell™ Plate with the standard tube-dispensing cassette, and 30 µl volumes into wells of 384-well optical bottom plates with the small tube plastic tip dispensing cassette. This resulted as cell concentration of 5000 cells per well in 96-well plates and 2000 cells per well in 384-well plates, respectively. The cells were automatically dispensed into columns 1-10 of the 96-well plates and columns 1-22 of the 384-well plates.

The same number of cells was added by hand-pipetting into the second-to-last column of the plates, and growth media was dispensed into the last column for the blank control. Cells were dispensed in four replicate plates for both plate types.

Cell viability assay

The viability of the cells after dispensing was tested with CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS). Before dispensing the cells, the AQ reagent was dispensed with the Multidrop Combi instrument and the small tube plastic tip dispensing cassette into the wells. The volume of the AQ reagent was 20 µl/well for the 96-well plates and 6 µl/well for the 384-well plates.

The plates were incubated for 2 hours at 37°C. The Thermo Scientific™ Varioskan™ Flash Multimode Reader was then used to record the absorbance at 490 nm.

Results

Visual examination

The adherent HeLa S3 cells were visually examined under the microscope before detaching the cells, and were found to be viable (figure 1a). The number of cells was then calculated after detachment and the visual estimation with the microscope was done again after dispensing the cells into the microplate with the Multidrop Combi dispenser (figure 1b). The cells in the suspension were viable and intact after dispense. The cells were further cultivated, and the cell culture adhered to the microplate and continued growing evenly (figure 1c).

Cell viability assay

The cell proliferation assay demonstrated that the levels of viability for the cells dispensed with the Multidrop Combi system and the manual pipette were the same.

Figure 2a) shows the evenness of dispense with the standard tube-dispensing cassette into the 96-well plate. The result of the column is the average value of the wells in one column, e.g. wells A01-H01.

Figure 2b) shows the same even distribution with the small tube plastic tip dispensing cassette. The average of the column is calculated from each column, e.g. A01-P01.

Cell viability is comparable to hand pipetting with both cassettes and plate.

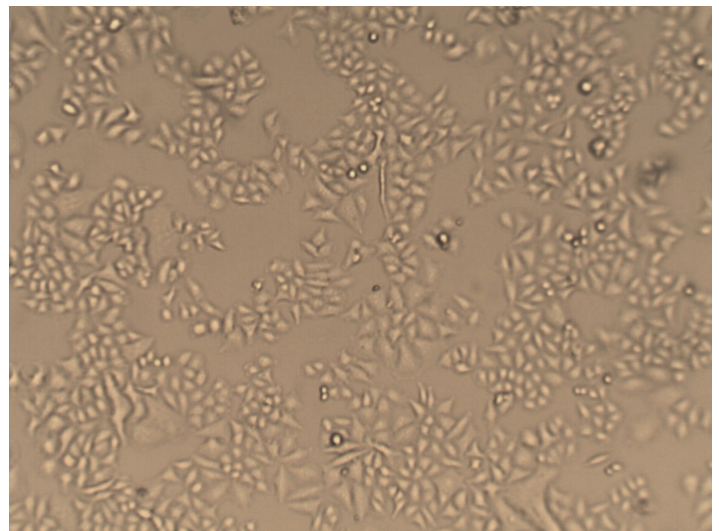


Figure 1a) HeLa S3 cells attached to the bottom of the cell culture flask

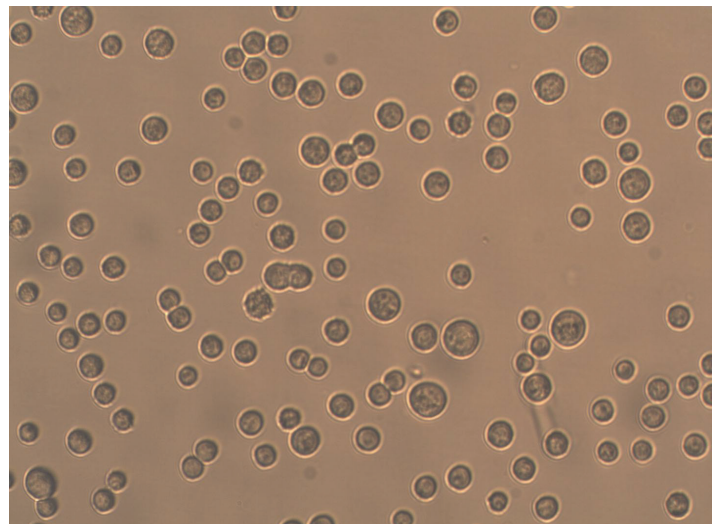


Figure 1b) HeLa S3 cells in the suspension immediately after dispense

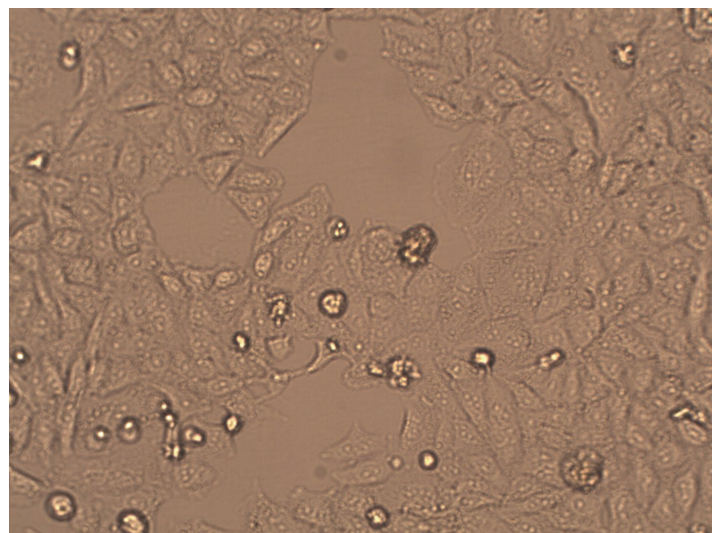


Figure 1c) Cells 24 hours after adhering to the 96-well plate bottom

Conclusions

The cell viability assay demonstrated that the Multidrop Combi dispenser is suitable for handling live cells. The cells remained intact and viable after dispense, and there were no traces of mechanical stress on the cells. Both the standard tube and small tube plastic tip dispensing cassettes show excellent precision for cell dispense.

The main issue to obtain reproducible cell dispensing results is the evenness of the cell suspension and the maintenance of the dispensing cassette to avoid any logging of the tubing or the tips.

References

1. Smart Tips - Best Practices: Dispensing Cells and Maintenance of Multidrop Dispensing Cassettes

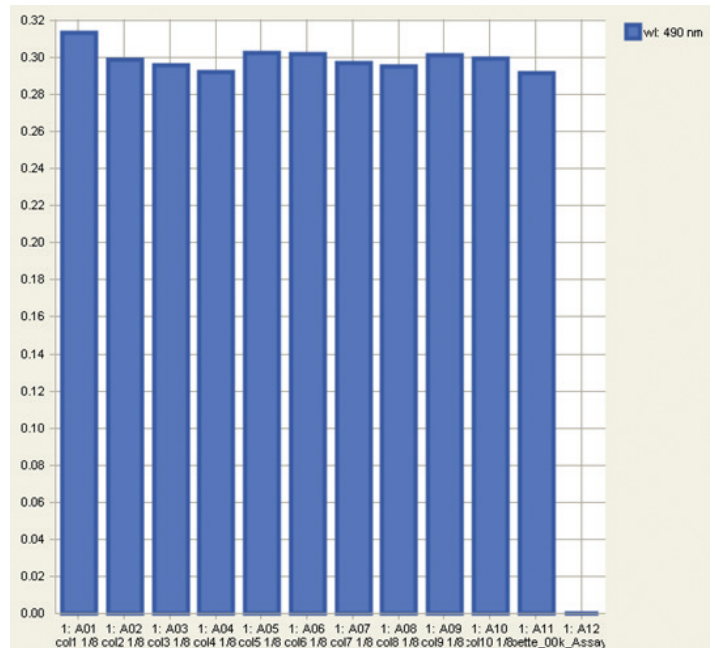


Figure 2. a) Dispensing of the cell suspension on the 96-well plate: A01-A10 Multidrop standard tubing, A11 hand pipetting, A12 blank control

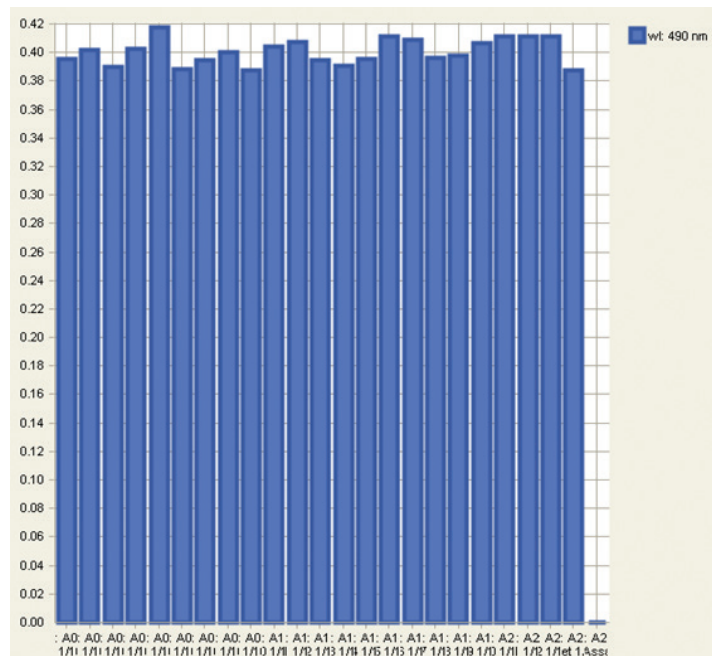


Figure 2. b) Dispensing of the cell suspension on the 384-well plate: A01-A22 Multidrop small plastic tubing, A23 hand pipetting, A24 blank control

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