

# Cell-Based Assays in 384 and 1536 Well Formats Using Mosquito® and Acumen Explorer™



Paul Wylie & Rob Lewis

TTP LabTech Ltd, Melbourn Science Park, Cambridge Road, Melbourn, Royston, Herts SG8 6EE, UK

## Abstract

There is growing interest in using whole cell assays for screening. By using a 1536 well format, the quantities of expensive compounds needed can be substantially reduced, having a major impact on the cost of a screen. A number of technologies can dispense cells into 384 well formats, but which are able to dispense viable cells into a 1536 well plate.

In this study, we used the Mosquito liquid handling system to dispense cells into 384 and 1536 well plates. We then determined cell cytotoxicity as an example of a whole cell assay. We also used the Acumen Explorer to study proliferation of the cells over several days.

The results demonstrate that Mosquito can successfully dispense viable cells into 384 and 1536 microtitre plates suitable for detection and assay with Acumen Explorer.

## Introduction

As interest in using whole cell assays for primary screening grows, researchers are turning to high density plate formats, including 1536 well, to meet screening demands. Currently, there have been problems in developing these assays due to limitations in successfully pipetting viable cells and the subsequent reading of assay plates.

In this study, we used the Acumen Explorer, a laser-scanning fluorescence microplate cytometer and Mosquito, a liquid handling system employing disposable micropipettes, to study cytotoxicity and proliferation in CHO cells and HL60 cells.

## Assay Summary

- Mosquito liquid dispensing
- Multiplexing: count live and dead cells per well
- High throughput: > 100,000 data points per day

## 1 Assay Protocol

- A 1536 or 384 well microtitre plate was prepared by adding 5  $\mu$ L or 45  $\mu$ L respectively of media.
- Cells were harvested by Trypsin/EDTA, triturated to break up clumps and resuspended to achieve the following cell numbers in 1  $\mu$ L.
- Using Mosquito, 1  $\mu$ L of cell suspension was added to each well in either plate type.

PlateType	Assay (cells/ $\mu$ L)	
	Proliferation	Cytotoxicity
1536	50	250
384	250	1000

### Proliferation Assay:

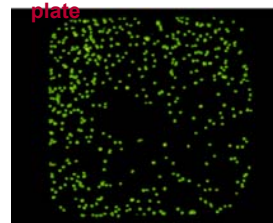
- Prior to each scan, 1  $\mu$ M of calcein (excitation 495 nm, emission 515 nm) was added to each well by Mosquito and the plate was incubated for 30 min @ 37°C / 5% CO<sub>2</sub>. The plate was then scanned on Acumen Explorer. Different sections of the plate were scanned at the time points indicated.

### Cytotoxicity Assay:

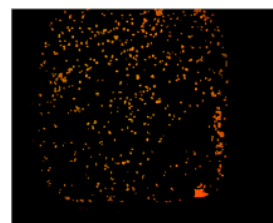
- Methanol was added and incubated for 30 min at @ 37°C / 5% CO<sub>2</sub>, followed by the addition of 1  $\mu$ M of calcein/ 2  $\mu$ M propidium iodide (excitation 535 nm, emission 617 nm). The plate was incubated for 30 min @ 37°C / 5% CO<sub>2</sub>. The plate was then scanned on Acumen Explorer.

Data shown is from 1536 well plates, however, all data is representative of that obtained in 384 well plates.

## 2 Cytotoxicity assay well view in Acumen Explorer of HL60 cells in a 1536 well plate

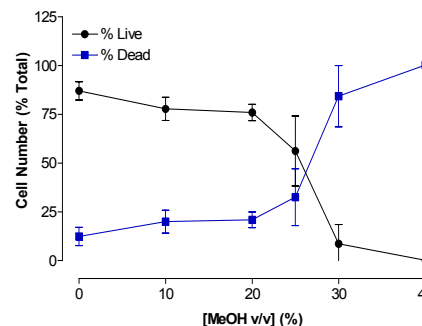


Live Cells (calcein staining) with 0% methanol

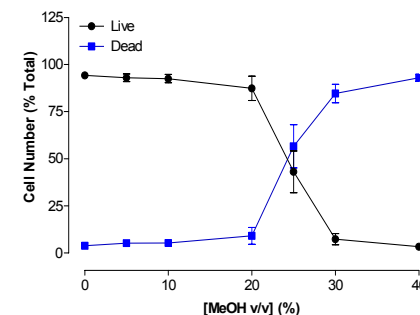


Dead Cells (propidium iodide staining) with 40% methanol

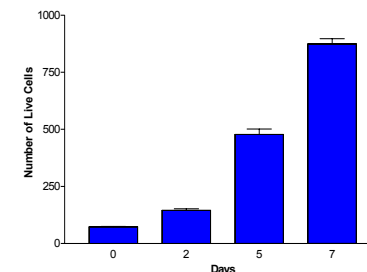
## 3 Effect of methanol on cell death in CHO cells in a 1536 well plate



## 4 Effect of methanol on cell death in HL60 cells in a 1536 well plate



## 5 CHO cell proliferation in a 1536 well plate in response to 10% FCS



## Conclusion

Using Acumen Explorer and Mosquito, we have shown that cell cytotoxicity and proliferation can be determined, as examples of whole cell assays.

The results demonstrate that Mosquito can successfully dispense viable cells into 384 and 1536 microtitre plates suitable for detection and assay with the Acumen Explorer.