A Novel High Throughput-Compatible Cell Migration Screening Assay using an Acumen eX3

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Abstract

Cell migration is a highly integrated, multi-step process. Dysregulated cell migration has been implicated in cancer, macular degeneration and diabetic wound healing. Understanding the processes involved in cell movement can provide important insight into the management of multiple disease states. Traditional methods to monitor 2-D closure have utilized scratch assays but these can produce inconsistent detection zones and are not readily compatible with high throughput screening strategies.

The Oris™ Cell Migration Assay utilizes unique cell-seeding stoppers to create a detection zone for visualizing cell migration and invasion. Silicone stoppers are inserted in each well of a 96-well plate, around which adherent cells form an annular pattern 4-18 hours after seeding. Removal of the stopper reveals a uniform 2 mm detection zone in the center of the monolayer into which the cells can migrate.

In this study the effect of blebbistatin, the cell-permeable actin-myosin inhibitor, on the migration of MDA-231 cells and HT-1080 cells was determined. Cell migration was quantified on the Acumen® eX3 microplate cytometer by using the Distance Object Characteristic feature. Using this instrument, the number of migrating cells was obtained and the EC_{50} values for blebbistatin were determined. The Acumen eX3 instrument was able to analyse a 96 well plate in approximately 7 minutes resulting in a throughput of greater than 800 data points per hour.

Analyzing the Oris™ Cell Migration Assay with the Acumen eX3 instrument yields reliable and highly accurate cell migration data with high throughput capability.

Conclusions

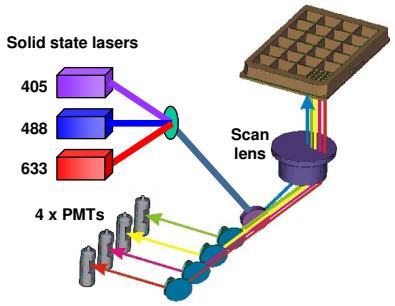
- Oris™ Cell Migration Assay-TriCoated plates are recommended for initial assay development studies
- Acumen eX3 is conducive for rapid data acquisition with analysis of Oris™ Cell Migration Assays within 10 minutes for a 96-well assay plate
- Oris™ Cell Migration Assays are conducive for multiplexed staining using different fluorescent labels
- Oris™ Cell Migration Assays are highly reproducible with Z' factors and signal:noise ratios that are acceptable for compound screening

References

Zhang JH, Chung TD, Oldenburg KR, "A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays." *J Biomol Screen.* 1999; **4**(2):67-73.

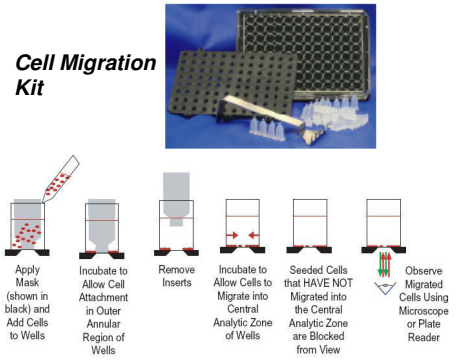
Hulkower K, Perr M, Quantifying Cell Migration and Invasion. *GEN*, 2008; **28**(17):pp32

1 Acumen eX3 Microplate Cytometer Optical Setup



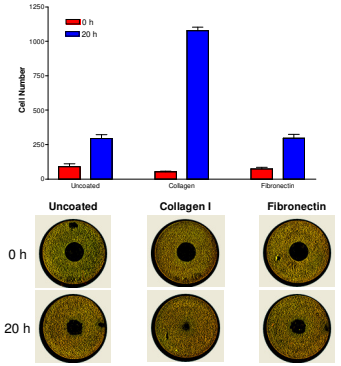
The Acumen eX3 can sequentially scan with up to 3 lasers providing similar wavelength excitation to that of white light sources. PMTs detect up to 4 colours simultaneously. The application of laser scanning over a large area means that analysis is performed on an area, not a well basis. This equates to the simultaneous scanning of 4, 16 and 64 wells in 96, 384 and 1536 well format, respectively. Thus reconfiguration of assays into higher density plate formats results in a concomitant increase in throughput up to 300,000 samples per day in 1536 well microplates.

2 Oris™ Cell Migration Assay



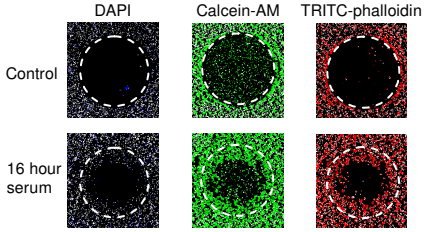
The Oris™ Cell Migration Assay is designed with a unique cell seeding stopper, detection mask, and stopper tool. There is no transmembrane insert is needed. This unique plate design generates highly reproducible results using a microscope, digital imaging system or fluorescence plate reader. Simply adhere the seeded cells in each well, remove the stopper to create a detection zone, and measure the migration. The Oris™ Cell Migration Assay is offered with stoppered plates having tissue culture treated surfaces (uncoated), coated with type I collagen or fibronectin. The Oris™ TriCoated plate has 32 wells of each surface on a single plate.

3 Migration of MDA-231 cells on Oris™ TriCoated plates



MDA-231 cells were seeded on Oris™ TriCoated assay plates with either no treatment, collagen I, or fibronectin coated wells. Cells were stained with DAPI (bar chart), Calcein-AM (well images) and TRITC-phalloidin (data not shown). The number of cells was determined pre- and post- migration with a 20 hour incubation time. The data show that using cell number, all three dyes gave the same results (data not shown). The use of collagen as opposed to uncoated or fibronectin coated plates gave the greatest degree of migration.

4 Quantification of Different Fluorescent Labels to Assess HT-1080 Cell Migration

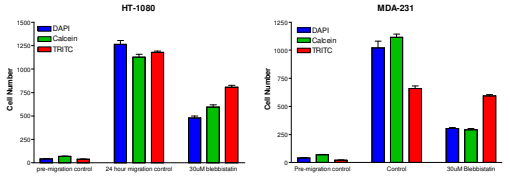


Dye	Z'	Fold Window
DAPI	0.48	19.03
Calcein-AM	0.50	22.79
TRITC-phalloidin	0.50	18.73

$$Z' = \frac{(\text{mean sample} - (3SD\ \text{sample}/\sqrt{n})) - (\text{mean control} - (3SD\ \text{control}/\sqrt{n}))}{\text{mean sample} - \text{mean control}}$$

The Acumen eX3 can detect migration using different stains using different lasers. This allows easy selection of other dyes in order to multiplex assays with other screening targets.

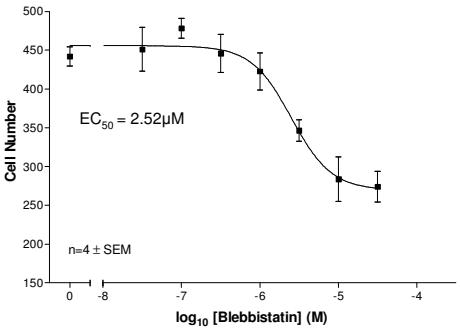
5 Quantification of Different Fluorescent Labels to Assess the Effect of Blebbistatin on Cell Migration



Dye	HT-1080 Z'	HT-1080 Fold Window	MDA-231 Z'	MDA-231 Fold Window
DAPI	0.72	26	0.77	30
Calcein-AM	0.88	16	0.70	12
TRITC-phalloidin	0.91	28	0.86	48

HT-1080 and MDA-231 cells were seeded on an Oris™ plate coated with type I collagen and allowed to migrate for 24 hours in the presence and absence of 30 µM blebbistatin. Cells were stained with either DAPI, Calcein-AM or TRITC-phalloidin. The number of cells within the defined area of interest were determined. Z' and Fold window values were calculated using pre-migration controls versus 24 hour migration controls (n=8). DAPI and Calcein-AM stains show similar results of migration inhibition by blebbistatin.

6 Dose Response Curve of Blebbistatin Treatment on HT-1080 Cell Migration



HT-1080 cells were seeded on a Oris™ tissue culture treated plate (uncoated) at 50,000 cells per well in FBS containing serum, and incubated with blebbistatin for 17 hours. The cells were then stained with Calcein-AM prior to scanning on the Acumen eX3.