



## APPLICATION NOTE

AN011-01

### To study the effects of a Rho Kinase Inhibitor on Horizontal Migration using the Cell-IQ®

#### Objective

– to investigate the effect of Y27632, a Rho Kinase inhibitor, on breast cancer cells in a wound-healing assay using the Cell-IQ®.

#### Material and methods

##### Cells

MDA-MB231 (available from ATCC), a breast cancer cell-line, plated out at  $2 \times 10^5$  cells/well

##### Growth Media

Dulbecco's MEM 9 (Gibco cat #)  
10% foetal calf serum  
1x Glutamine (Gibco 100x stock)

Y27632 – Merck BioSciences

#### Method

Cells were first incubated for 48 hours in Nunclon 48 well tissue culture plates at 37°C to enable attachment and growth to confluence. Less HT1080 cells were used per plate because of the different rates of proliferation – the numbers were optimised to provide confluence over the 48 hour incubation period. A 200 µl pipette tip was used to scrape an area of cells away from the confluent monolayer to mimic a wound. The media was changed with growth media + DMSO, or growth media + different concentrations of Y27632. The plates were then loaded onto the Cell-IQ® and independent areas for measurement were found in each well by scanning the plate manually. 3 Regions of Interest (ROI) per well per wound edge were chosen for imaging over a 21 hour period (1,2).

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## Results

### MDA-MB231

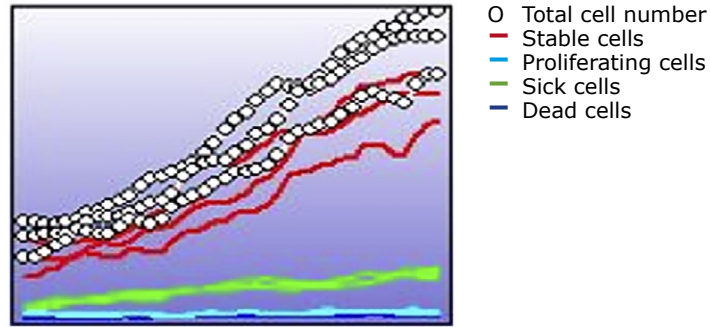


Fig 1. Cell-IQ® representation of triplicate data of MDA-MB231 cells (control) over a 21 hour test – showing steady increase in cell number over time. 50% of the wound is covered by migrating cells after 10 hours, and almost full wound closure is observed after 20 hours

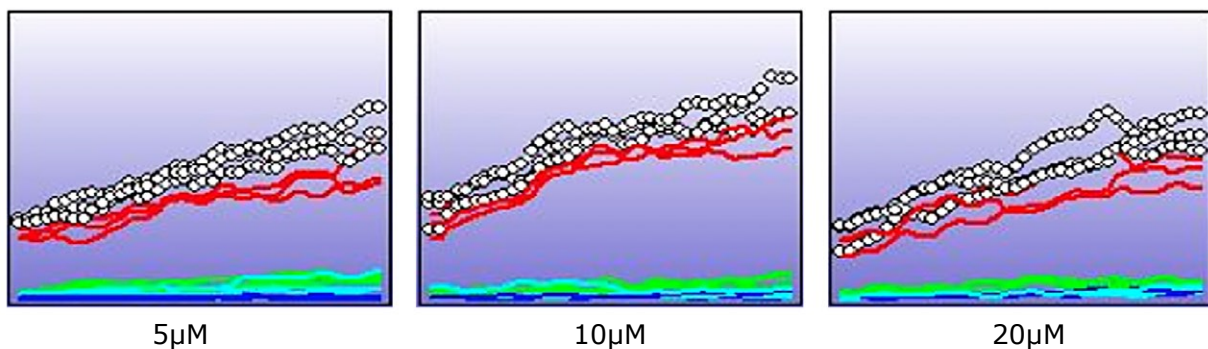


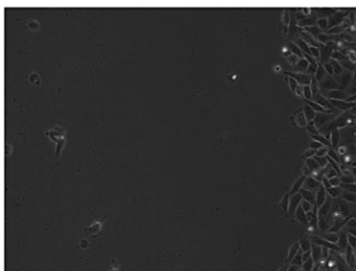
Fig 2. Numerical data representing triplicate results for each of the different concentrations of Y27632. The figures below represent those numbers, taken from each of the 3 ROIs within each well, to show the consistency of the analysis.

## Morphology changes

These are images of the culture during incubation and real-time image capture in the Cell-IQ® system. The Analyser software was trained to recognise the different cell types and measure the number of cells in the ROI at each time point.

In the presence of Y27632, the morphology of the MDA-MB231 cells changes, especially at the higher concentrations, and cytoplasmic pseudopods are very much in evidence.

### MDA-MB231



### MDA-MB231 + Y27632

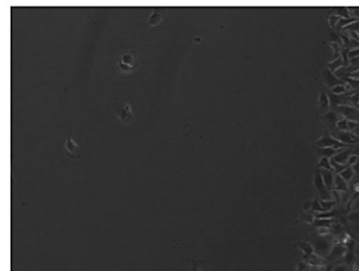
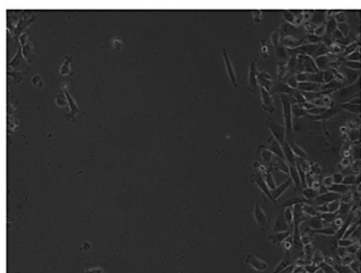


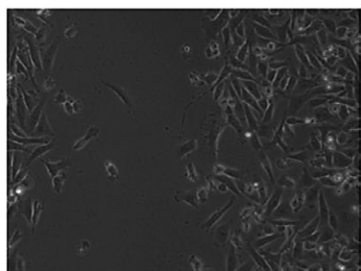
Fig 3. Cells at beginning of test

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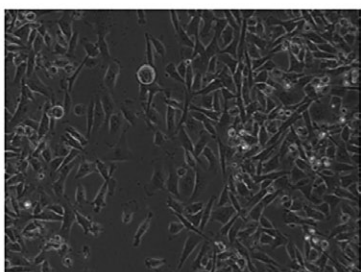
## MDA-MB231



*Fig 4. Cells at 4 hours*

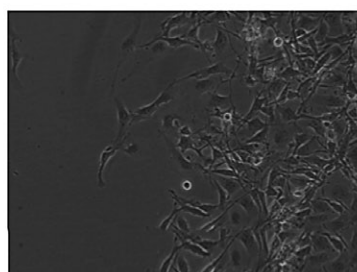
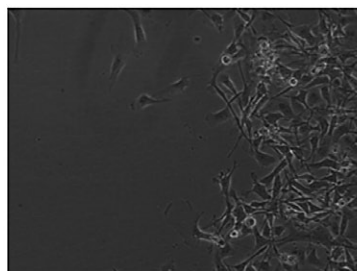
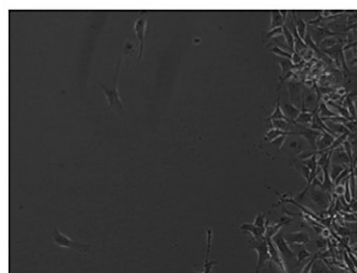


*Fig 5. Cells at 10 hours*



*Fig 6. Cells at 20 hours*

## MDA-MB231 + Y27632



## Summary

- Cell-IQ® provides a very fast and efficient way to measure the rate of migration of cells into a wound space. The simple output gives information on the number of cells moving into the denuded area of the well, as well as the number of cells proliferating and dying during the culture.
- Many replicates can be measured simultaneously – here we have used triplicate measurements – to give greater representation of the culture as a whole, whilst also giving information on reproducibility and reliability of your assay.
- The Cell-IQ® could also be used to generate comparative data on the change in rate of migration in the presence of potential drug candidates, whilst simultaneously monitoring cell proliferation and death.
- It was also observed that the cytoplasmic pseudopods originate at the back of the cell, and trail behind it as it migrates – this information would not be so evident using more traditional methods of time-lapse microscopy. With the Cell-IQ®, we can also measure the length of these protrusions.
- Kinetic profile is important for compound analysis and assay development (optimal time-points for analysis for HTS)

## References

- (1) Data by kind permission of N.Carragher, Enabling Science & Technology Group, Astra-Zeneca R&D Charnwood, Loughborough, UK.
- (2) L.Scott et al, Molecular and Cellular Biology, Feb 2004, p1540-1559, Vol 24, No4.