# A high-throughput colony formation assay for profiling novel compounds and RNAi reagents using the Acumen® eX3

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## Introduction

Cell colony formation assays measure a cell's ability to grow unattached to a surface and have applications in a range of areas including hematopoietic stem cell research, cell transformation studies and the prediction of responses of tumors to chemotherapeutic agents.

Traditionally, these assays have been carried out using a semisolid agarose bi-layer system in low density culture plates or petri-dishes with manual enumeration of colonies using a microscope. These methods are both low throughput and based upon subjective determination of the number of colonies thus making them unamenable to high throughput screening.

The objective of this study was to demonstrate that TTP LabTech's Acumen platform could quickly and reproducibly quantify the effects of test compounds and RNAi reagents on their ability to affect cell colony formation, and therefore be used as a screening platform.

A549 cells, growing in an agarose gel were incubated for 8 days in the presence of staurosporine, RNAi reagents and respective controls. The assessment included studying the effect of varying initial cell number and output parameters on the quality of the data.

It was shown that colonies seeded at 1,000 cells per well and stained with propidium iodide generated robust data. Using TTP LabTech's proprietary spherical volume algorithm, the effects of the staurosporine on the colony formation showed a concentration dependent inhibition of cell colony formation; RNAi treatments 2, 3 and 4 caused a large inhibition compared to the control, RNAi 1, all of which were statistically significant from the respective controls.

The results of this study demonstrated that the Acumen eX3 can be used as a high-throughput platform for investigation of effects of test compounds and RNAi reagents on cell colony formation.

## 1. Methods

The study was conducted using 96-well flat bottomed microplates. All wells were coated with a layer of 1.2% agar, A549 cells were suspended in 1.2% agar solution and added to each well at densities of 500, 1,000 and 1,500 cells per well (see Figure 1).

Staurosporine (0.1nM – 1µM), staurosporine vehicle (DMSO), four RNAi reagents and a no treatment control (NTC) were added to the wells according to Figure 1. All treatments were incubated for 8 days at @ 37°C/5% $CO_2$. At the end of the incubation period all plates were fixed with 4% formaldehyde. All cells were stained with 1.5µM propidium iodide (PI) and scanned using an Acumen eX3. Data was analysed using TTP LabTech's proprietary spherical volume algorithm.

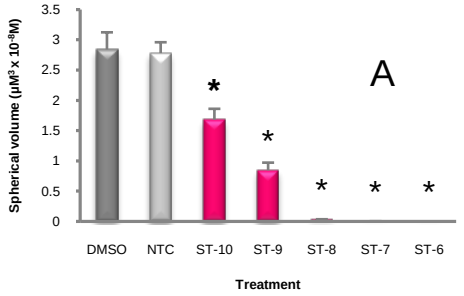
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | ST-10 | ST-10 | ST-10 | DMSO | ST-10 | ST-10 | ST-10 | DMSO | ST-10 | ST-10 | ST-10 | DMSO |
| B | ST-9 | ST-9 | ST-9 | DMSO | ST-9 | ST-9 | ST-9 | DMSO | ST-9 | ST-9 | ST-9 | DMSO |
| C | ST-8 | ST-8 | ST-8 | DMSO | ST-8 | ST-8 | ST-8 | DMSO | ST-8 | ST-8 | ST-8 | DMSO |
| D | ST-7 | ST-7 | ST-7 | NTC | ST-7 | ST-7 | ST-7 | NTC | ST-7 | ST-7 | ST-7 | NTC |
| E | ST-6 | ST-6 | ST-6 | NTC | ST-6 | ST-6 | ST-6 | NTC | ST-6 | ST-6 | ST-6 | NTC |
| F | RNAi - 1 | RNAi - 2 | RNAi - 3 | RNAi - 4 | RNAi - 1 | RNAi - 2 | RNAi - 3 | RNAi - 4 | RNAi - 1 | RNAi - 2 | RNAi - 3 | RNAi - 4 |
| G | RNAi - 1 | RNAi - 2 | RNAi - 3 | RNAi - 4 | RNAi - 1 | RNAi - 2 | RNAi - 3 | RNAi - 4 | RNAi - 1 | RNAi - 2 | RNAi - 3 | RNAi - 4 |
| H | RNAi - 1 | RNAi - 2 | RNAi - 3 | RNAi - 4 | RNAi - 1 | RNAi - 2 | RNAi - 3 | RNAi - 4 | RNAi - 1 | RNAi - 2 | RNAi - 3 | RNAi - 4 |
| | 500 cells per well | | | | 1000 cells per well | | | | 1500 cells per well | | | |

Figure 1 – Plate map. Each plate was divided into three sections corresponding to plating densities of 500, 1,000 and 1,500 cells per well respectively. Staurosporine (0.1nM – 1µM), staurosporine vehicle (DMSO), four RNAi reagents and a no treatment control (NTC) were tested in triplicate.

## 2. Results

The data from the wells seeded at 1,000 cells per well exhibited the least variability across the replicates when compared to the other plating densities (data not shown). Multiple parameters were compared and total area and spherical volume were found to be in concordance, with spherical volume giving the largest fold change between control and treatments (data not shown).

All treatments were statistically analysed using a one-way ANOVA, followed by a Dunnett's multiple comparison test. Analysis of the staurosporine treatments showed staurosporine inhibited cell colony formation in a statistically significant and concentration-dependent manner, with complete inhibition being observed in the range 10nM – 1µM, all treatments were significantly different from the DMSO control, only the NTC was not different from the DMSO control (see Figure 2, Panel A). Analysis of the RNAi data found that RNAi 2, 3 and 4 were statistically different from RNAi 1 (non-silencing control) (see Figure 2, Panel B). Representative TIFF and cytometric images generated by the Acumen can been seen in Figures 3 and 4. Figure 3 shows a direct comparison of a TIFF and cytometric image from a control well. Figure 4 shows TIFF images demonstrating the effect of staurosporine and one of the RNAi reagents.



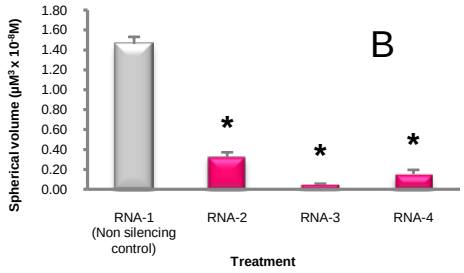




Figure 2 – Inhibition of colony formation by staurosporine and RNAi treatment. Cell colony size is expressed as total spherical volume of colonies within each well. Colonies are stained with 1.5µM PI. The initial seeding density was 1,000 cells/well. Panel A, staurosporine treated wells (DMSO control, NTC-none treated control, ST-10, 0.1nM staurosporine etc). Following an ANOVA (P<0.0001) for the staurosporine data, each group was compared to the DMSO control group, using Dunnett's multiple comparison test. All staurosporine treatments were significantly different from the DMSO control, only the NTC was not different from the DMSO control. Panel B, Analysis of the RNAi data (ANOVA = P<0.0001) found that RNAi 2, 3 and 4 were statistically different from RNAi-1 (non-silencing control) (Dunnett's multiple comparison test). * denotes statistically significant differences (p<0.001; Dunnett's multiple comparison test).

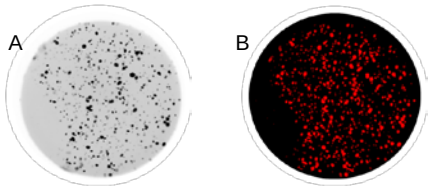



Figure 3 – Colony view of well F9, PI stain. Panel A shows a TIFF image and Panel B a cytometric image. Both images were generated using the Acumen software.

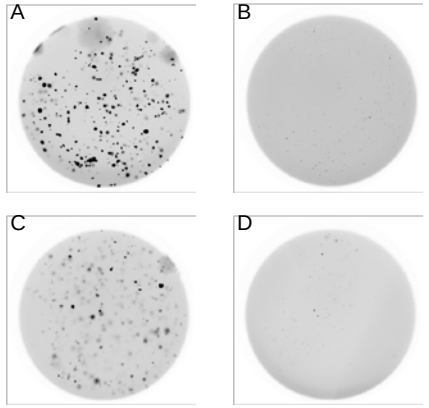
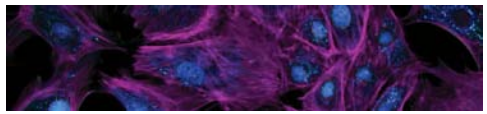



Figure 4 - Representative TIFF images from a PI stained plate showing colony formation in the presence of test treatments. Panel A, well C8 – DMSO, Panel B, well C7 - staurosporine 10nM, Panel C, well F5 – RNAi-1 non-silencing control, Panel D, well F7 – RNAi 3. All wells seeded at 1,000 cells/well.

## Conclusion

- Determination of total spherical volume using TTP LabTech's proprietary algorithm provides a robust measure of effects on colony formation.
- To achieve a stable and reproducible assay a plating density of 1,000 cell per well was found to be optimal. This provided sufficient cells to show clear effects on cell colony formation as detected using the Acumen eX3.
- The Acumen eX3 provides a fast and reproducible platform for the investigation of test compounds and RNAi reagents on cell colony formation, offering an alternative to traditional lower throughput manual techniques.



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