Determination of Cell Colony Formation in a High Content Screening Assay

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Abstract

Cell colony formation assays are used extensively for research and clinical applications to assess the functional integrity of cells after *in vitro* manipulations. Key areas include haematopoietic stem cell research, cell transformation studies and predicting the response of tumours to chemotherapeutic agents. Traditionally, enumeration of colonies has involved laborious counting by hand using a microscope. Here, laser scanning microplate cytometry has been used to provide an automated high content readout of the effects of cytostatic agents on colony formation. This approach determines colony number through the application of a spherical volume algorithm. This permits the differentiation of cytostatic effects where the number of colonies and size remains constant and cytotoxic effects where the size and number may be reduced. Application of microplate cytometry thus offers significant benefits over alternative methods in the search for novel chemotherapeutic agents.

Introduction

Cell colony assays are used extensively for research and clinical applications to assess the functional integrity of cells after *in vitro* manipulations. Cell colony formation has been found to be a more sensitive parameter of toxicity than cell viability as colony formation is assessed while the cells are in a state of proliferation, and more susceptible to toxic effects.

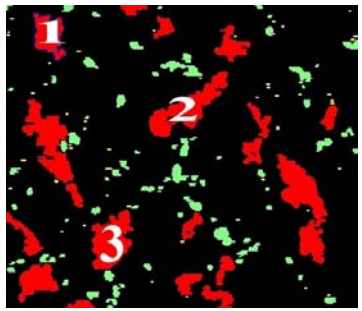
Enumeration of colonies has been traditionally carried out using a semisolid agarose bilayer system in petri dishes and involved laborious counting by hand on a microscope. Recent advances have provided automatic number counting of bacterial colonies using digital colour cameras which with the accompanying software allows a number of different colony colours to be distinguished. More advanced image analysis systems only image in 2D and have difficulty in distinguishing accurately, large voluminous colonies, due to the limited depth of field. In addition they are unable to offer cell counting on a whole well basis due to the imaging of small well areas, typically less than 1mm x 1mm. The Acumen Explorer™ laser scanning microplate cytometer is a non-confocal system, permitting area-based scanning of fluorescent objects in whole wells, without the need of a nuclear stain required by image platforms. It also does not need to re-focus between wells enabling high scan speeds on any SBS standard plate.

Here, the Acumen Explorer™ has been used to provide an automated high content whole well readout from 24 well plates of the effect of a cytostatic agent staurosporine on colony formation of HT1080 cells. The software determines colony number through the application of a spherical volume algorithm.

1 Assay Protocol

- In a 24 well tissue culture plate place a 300µL base layer of 0.5% agarose by adding autoclaved 1% agarose solution to 2x DMEM medium with 20% FCS in a ratio of 1:1.
- Harvest HT1080 cells and make up to 2.5 x10^6 cells/mL. Add 0.1mL to 10 mL of a 1:1 mixture of 0.7% agarose solution and 2x DMEM with 20% FCS.
- Add 400µL to the top of the base layer, allow to solidify then place in 37°C/ 5% CO_2 incubator.
- The following day add 400µL of a 2x test compound dilution in media to appropriate wells.
- Microscopically monitor cultures for colony formation and when the appropriate size is reached, stain with 0.5µM calcein.
- Scan on the Acumen Explorer.
- Locate colonies of the desired cell number, in control wells, using a microscope. Position these colonies in the Well View on the Acumen Explorer and use the spherical volume values to classify the minimum size of colonies.

2 Identification of colony formation of HT1080 cells



Colonies containing more than 20 cells were identified and classified as a colony. In Figure 2, colonies (red) are easily discriminated from clusters of less than 20 cells (green). The spherical volume algorithm also accounts for different shaped colonies, as labelled 1, 2 and 3.

3 Cell colony formation of HT1080 cells on the Acumen Explorer

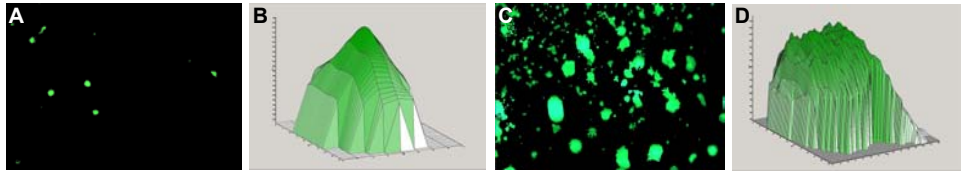
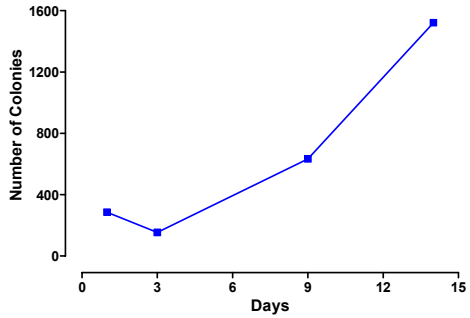


Figure 3A is an Acumen Explorer well view showing individual HT1080 cells at day 0. Figure 3B shows a corresponding fluorescent profile of a single cell.

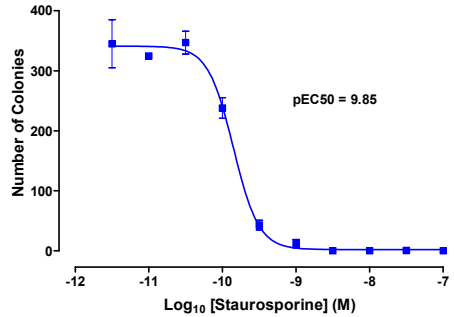
At day 14, cell colonies have formed. Figure 3C shows a well view of HT1080 colonies, Figure 3D is a fluorescent profile of a cell colony.

4 Time course of HT 1080 cell colony formation



Time course of colony formation by HT1080 cells. (data are representative of results obtained from 3 separate experiments).

5 The effect of staurosporine on HT1080 cell colony formation



Concentration-dependence curve of staurosporine-induced inhibition of HT1080 colony formation after 12 days. (data are representative of results obtained from 3 separate experiments).

Conclusion

We have successfully used the Acumen Explorer with a spherical volume algorithm to determine cell colony formation. This method allows rapid enumeration of colony number suitable for higher throughput compound assessment than current microscope-based methods. We have determined the time course of HT1080 colony formation in a 24 well plate and its inhibition by staurosporine. The assay provides a simple, high content and high throughput method for predicting the response of tumours to chemotherapeutic agents.

References

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