A multiplexed proliferation screening assay: Differentiation and measurement of proliferation profiles in a mixed cell population using quantum dots

Paul Wylie[1], John Budd[1] and Marcel Bruchez[2]
[1]TTP LabTech Ltd, Melbourn Science Park, Melbourn, Hertfordshire, UK
[2]Quantum Dot Corporation, 26118 Research Road, Hayward, CA 94545

QUANTUMDOT

Abstract

The development of multiplexing capabilities and high content readouts reporting individual cellular measurements enables assessment of biological variability on a single cell basis, together with evaluation of cell sub-populations within wells. A high content screening multiplexed assay format allows additional information to be gained from a single assay. One such example is the ability to determine effects of new chemical entities on different cell lines, tested in the same well. These assays, coupled with an appropriate automated cell-analysis platform, enable scalable screening of compound libraries for selectivity or toxicity. This approach can greatly increase screening efficiencies and enhance the amount of information achieved from a particular assay procedure, resulting in a significant reduction in the overall cost of a chemical compound library screen. We have used the unique optical properties of semiconductor quantum dots to achieve in-well multiplexing of distinct cell populations. By labeling two cell lines with different colored live cell markers, we have determined the differential rates of cell proliferation of the individual cell lines in a single well. This approach can extend to more than two cell populations and to measure drug-induced differential changes in proliferation in a single well assay on multiple cell lines.

Introduction

Qtracker™ reagents deliver fluorescent Qdot® nanocrystals into the cytoplasm of live cells using a custom targeting peptide[1,2]. Once inside the cells, Qtracker labels provide intense, stable fluorescence that can be traced through several generations and are not transferred to adjacent cells in a population. Labeled cells can be observed for many days, with no cytotoxic, photobleaching, or degradation problems commonly associated with fluorescent dyes[3,4]. Qtracker is therefore, an excellent tool for long-term studies of live cells for example in studying the proliferation of different cell lines in the same well by multiplexing several colors. Qtracker labels provide a powerful means to understand the complexity and dynamics of biological interactions of multiple live cells simultaneously.

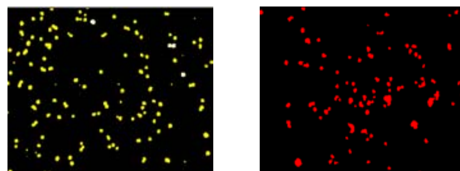
Acumen Explorer™ combines the sensitivity of image-based systems with the high read speeds of traditional bulk readers. At the heart of the system is a proprietary, non-confocal optic system which permits focus-free, area-based scanning, collecting up to 4 channels of data in a single scan. To prevent problems of variable cell number, patchy stimulation and edge effects, Acumen Explorer allows the researcher to define the scan area within each well. Scan options include the whole well area, centre well or user-defined strips. This gives the user the ability to scan 96, 384 and 1536 well plates in the same time

Here, we show a HTS-compatible multiplexed proliferation assay which can be run at plate read times of 5 minutes per 384 well plate We have investigated the effects of culture media on the proliferation of CHO and SH-SY5Y cells. The different proliferation rates of these cells have been investigated as single populations and as a mixed cell population. This is possible by virtue of fact that Qtracker can be traced through several cell generations and that the Acumen Explorer can analyse the same well over time without losing sterility.

1 Assay Protocol

SH-SY5Y and CHO cells were routinely passaged in DMEM and MEM alpha media respectively, and plated out at 20,000 cells per well into a 24 well plate. Cells were allowed to adhere overnight. The following day, the CHO cells were labelled with Qtracker 655 Kit and SH-SY5Y cells with Qtracker 705 Kit, then incubated for at least 1 hour to overnight. The cells were lifted from the 24 well plate and plated into a 96 or 384 well plate from between 500-2000 cells per well in the media indicated. The cells were scanned using the Acumen Explorer on the days shown to determine cell number. Cells were distinguished from debris using size classifiers. The data shown are from 96 well plates, although similar results were obtained from 384 well plates.

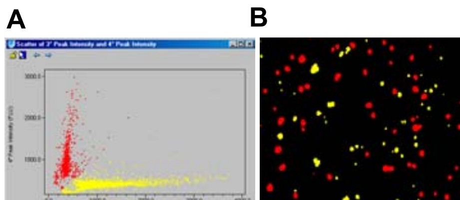
2 Well View of CHO and SH-SY5Y cells



CHO cells labelled with 655nm Qtracker reagents.

SH-SY5Y cells labelled with 705nm Qtracker reagents.

3 Mixed population of CHO and SH-SY5Y cells

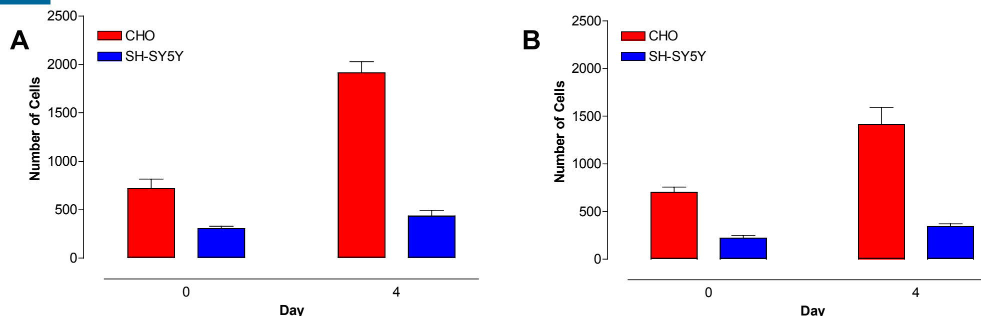


By simultaneously scanning for both Qtracker reagents, the ratio of 655nm to 705nm fluorescence can be quantified and CHO cells can be discriminated from SH-SY5Y cells in the same well.

A. Scatter chart of mixed cell population. SH-SY5Y cells (red), and CHO cells (yellow).

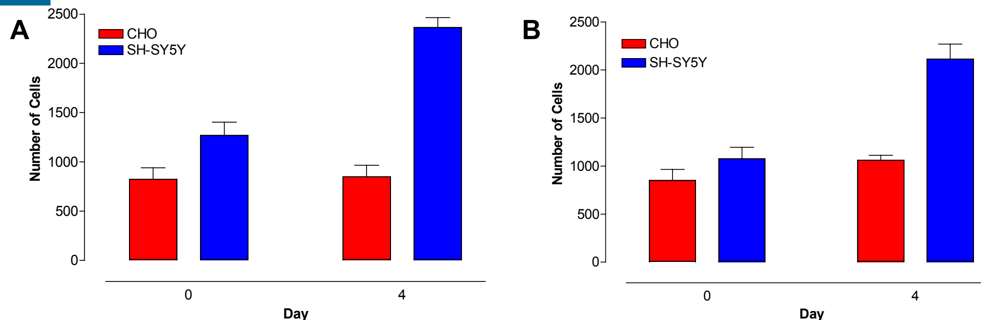
B. Well view of a mixed cell population, showing CHO and SH-SY5Y cells together in the same well after 4 days culture.

4 Proliferation of CHO and SH-SY5Y cells in CHO media (MEM-alpha)



Proliferation of CHO and SH-SY5Y cells grown in CHO media in separate wells (**A**) and in a mixed cell population (**B**). Data represent means ± S.D. of 4 replicates and are representative of results obtained from 3 separate experiments.

5 Proliferation of CHO and SH-SY5Y cells in SH-SY5Y media (DMEM)



Proliferation of CHO and SH-SY5Y cells grown in SH-SY5Y media in separate wells (**A**) and in a mixed cell population (**B**). Data represent means ± S.D. of 4 replicates and are representative of results obtained from 3 separate experiments.

Conclusion

We have shown that by labelling live cells with Qtracker™ reagents and identifying cells over time using the Acumen Explorer, we were able to determine different proliferation effects in distinct cell types in response to exposure to different media. The data show that the same proliferation effects were observed in both single and mixed cell populations. The data show CHO cells are quiescent in SH-SY5Y media, and SH-SY5Y cells are quiescent in CHO media. We were also able to analyse the same wells at day 0 and day 4, thus obtaining data from the same wells, without the need to use different wells for each time point. These results have been shown in both 96 and 384 well plate formats.

References

1 Jaiswal, J. K. *et al.* (2003). *Nature Biotech.* **21:** 47-51.

2 Hanaki, K. *et al.* (2003). *Biochem. Biophys. Res. Comm.* **302:** 496-501.

3 Futaki, S. (2003). *Curr. Protein Pept. Sci.* **4**(2)**:** 87-96.

4 Umezawa, N. *et al.* (2001). *J. Am. Chem. Soc.* **124:** 360-369.

TTP LABTECH

TTP LabTech Ltd., Melbourn Science Park, Melbourn, Royston, Herts SG8 6EE UK. Phone +44 (0)1763 262626 Visit us at www.ttplabtech.com/explorer

Quantum Dot Corporation, 26118 Research Road, Hayward, CA 94545 USA. Phone (510)-887-8775 Visit us at www.qdots.com