Application Note

Cell Analysis

Agilent

Trusted Answers

Real-Time and Dynamic Monitoring of Cell Proliferation and Viability for Adherent Cells

xCELLigence real-time cell analysis

Author

Agilent Technologies, Inc.

Introduction

The use of cell-based assays is crucial for understanding the efficacy, permeability, solubility, and stability of drug interaction with target cells. The data collected from cell-based assays are also pertinent to the process of drug discovery. It can help determine whether a given drug should proceed to the next phase in development, such as animal studies and ADME (absorption, distribution, metabolism, and excretion) analysis. Researchers must therefore choose the right assay platform for their cell-based assays if they are to exploit the full potential of their compound libraries.

Most assay formats designed for analysis of cell proliferation and viability are single endpoint assays. Techniques commonly used include:

- WST-1 assay
- XTT, MTT assay
- BrdU assay
- Fluorescence microscopy

Such assays have several limitations. First, they are labor-intensive and involve labeling steps in addition to a lysis or fixation step. These assays also suffer from a lack of quality control when used to determine cell viability before and after treatment. Determinations are important for ensuring consistency and reproducibility in the same assay or between different assays. The assays also provide limited information about the mechanism of drug interaction with the target cells. Finally, since the detection method for most of these assays is based on optical methods, some compounds and cells tend to interfere with the method of detection and can obscure the data.

The xCELLigence system was developed to allow label-free, dynamic monitoring of cell proliferation and viability in real time. xCELLigence instruments use an electronic readout called impedance to noninvasively quantify adherent cell proliferation and viability in real time. The cells are seeded in standard microplates that contain microelectronic sensor arrays. The interaction of cells with the electronic biosensors generates a cell-electrode impedance response that indicates cell viability and correlates with the number of the cells seeded in the well.

This impedance technology offers an innovative solution to various limitations in current assay systems. First, impedance measurements are noninvasive; the cells remain in a more normal physiological state during assays of cell proliferation and cytotoxicity. Second, the system is more economical because it does not require labeling the cells with expensive reagents. Finally, it allows real-time, rather than endpoint, measurement of cell proliferation, viability, and cytotoxicity.

Real-time, continuous monitoring offers several distinct advantages. It provides constant quality control for the cells, allowing users to make informed decisions about the timing of certain manipulations, such as compound addition. A kinetic record of the cells before or after a certain manipulation also provides important information about the biological state of the cell. This can reveal enhanced cell growth, cell quiescence, morphological changes, or cell death. When cytotoxic drugs are tested, real-time monitoring allows the calculation of time-dependent IC_{50} values. These values are more informative than single time-point IC_{50} values, which can vary dramatically depending on the time the experiment was terminated. Traditional methods would require multiple assays and experiments to clearly elucidate the mechanism of action of various drugs.

Several experiments were conducted to assess cell proliferation and drug interaction with different cell lines. This demonstrated that the new method is better suited for cell proliferation, viability, and cytotoxicity studies than traditional methods.

Materials and methods

Cells

All cells used in this study were obtained from ATCC and maintained in an incubator at 37 °C with 5% CO_2 saturation. H460, HepG2, and HT1080 cells were maintained in RPMI media containing 5% FBS, 1% penicillin, and 1% streptomycin. NIH 3T3 cells were maintained in DMEM media containing 10% FBS, 1% penicillin, and 1% streptomycin.

Cell proliferation assays

For each cell type, the indicated number of cells per well was seeded into 100 µL of media in 96X microplates (E-Plate). The attachment, spreading, and proliferation of the cells were monitored every 30 minutes using the xCELLigence system. Cell proliferation was monitored for 48 to 72 hours, depending on the experiment. Cell-sensor impedance was expressed as an arbitrary unit called the Cell Index (CI). The CI at each time point was defined as $(R_n - R_b)/15$, where R_n was the cell-electrode impedance of the well when it contains cells, and R_b was the background impedance of the well with the media alone.

Drug treatment and cytotoxicity assessment

The proliferation pattern of each cell type was used to determine its optimum cell concentration (Figure 1). The indicated number of cells per well was seeded into 100 µL of media in a 96-well E-Plate. The attachment, spreading, and proliferation of the cells were monitored every 30 minutes using the xCELLigence system.

Approximately 24 hours after seeding, when the cells were in the log growth phase, they were treated with 100 µL of the indicated compounds dissolved in cell culture media. The cells were also treated with DMSO, which served as vehicle control. Depending on the experiment, the final DMSO concentration in the media was between 0.25 and 0.5%.

MTT assay

Increasing numbers of NIH 3T3 cells were seeded into a 96X E-Plate and monitored with the xCELLigence system to obtain the corresponding Cell Index. The media was immediately aspirated and the cells were assayed using a standard MTT assay, according to the manufacturer's protocol.

Flow cytometry

A549 cells were seeded at a density of 500,000 cells per well in 60 mm tissue culture dishes. Approximately 24 hours after seeding, the cells were treated with the indicated final concentration of olomoucine. Sixteen hours later, the cells were washed with PBS, trypsinized, washed twice with PBS, fixed in 70% methanol, and stored at 4 °C until the staining step. The cells were stained with propidium iodide and analyzed by FACS at a wavelength of 488 nm.

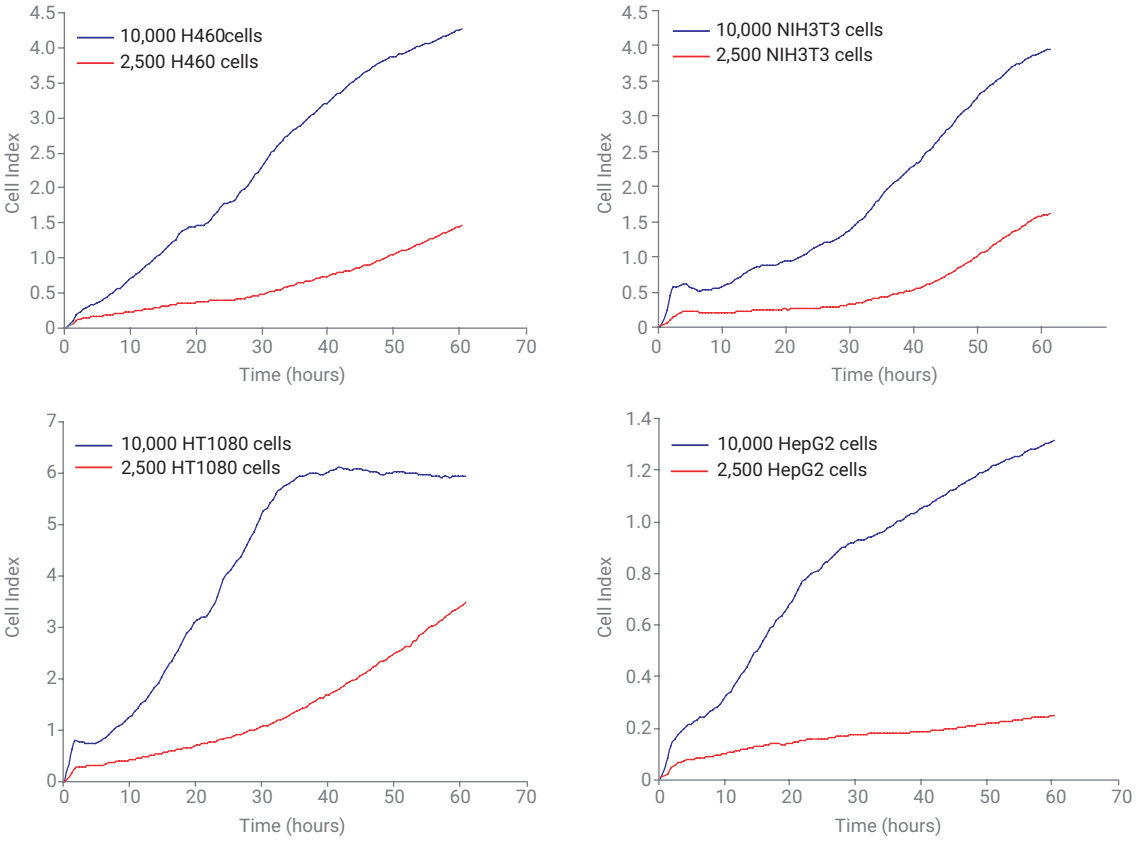

Figure 1. Dynamic monitoring of cell proliferation using impedance technology. H1080 fibrosarcoma cells, H460 lung cancer cells, HepG2 hepatosarcoma cancer cells, and NIH 3T3 mouse fibroblast cell lines were seeded at a density of 2,500 and 10,000 cells per well in 96X E-Plates. The adhesion, spreading, and proliferation of the cells were dynamically monitored every 30 minutes using the xCELLigence system.

Results and discussion

Monitoring dynamic cell proliferation in real time using impedance technology

H460 human lung cancer cells, H1080 fibrosarcoma cells, HepG2 human hepatosarcoma cells, and NIH 3T3 mouse fibroblasts were seeded at 2,500 and 10,000 cells per well in triplicate in 96-well E-Plates to assess dynamic cell proliferation. The cells were monitored every 30 minutes for the indicated time (Figure 1). As shown in Figure 1, each cell type has its own characteristic kinetic trace, based on the number of cells seeded, overall size and morphology of the cells, and degree to which the cells interact with the sensor surface. Also, each of the cell lines can be characterized by its unique adhesion and spreading kinetics, as well as the time at which it enters the log growth phase. Therefore, these parameters offer an excellent internal control, as well as a way to standardize and validate stock cultures during different phases of the manufacturing process.

Correlation between Cell Index units and number of cells seeded and comparison to MTT assay

Increasing numbers of NIH 3T3 cells were seeded into 96-well E-Plates to investigate whether Cell Index units correlate with the number of cells in the well. These were monitored for up to 10 hours, and the CI was obtained at the 10 hour time point. Figure 2A shows a plot of cell number seeded versus the CI obtained. In this experiment, the system detected as few as 100 NIH 3T3 cells, and the readout was linear over two orders of magnitude up to 10,000 cells.

At the end of the experiment described in Figure 2A, the cells were assayed with the MTT assay. As shown in Figure 2B, up to 1,000 cells produced MTT assay values that were not above background. For cell numbers exceeding 1,000, the MTT units show a linear correlation with the number of cells seeded.

While impedance technology is capable of dynamic and continuous measurements, for the experiment in Figure 2, impedance was only measured at a single point so the results could be compared with the MTT single-point assay.

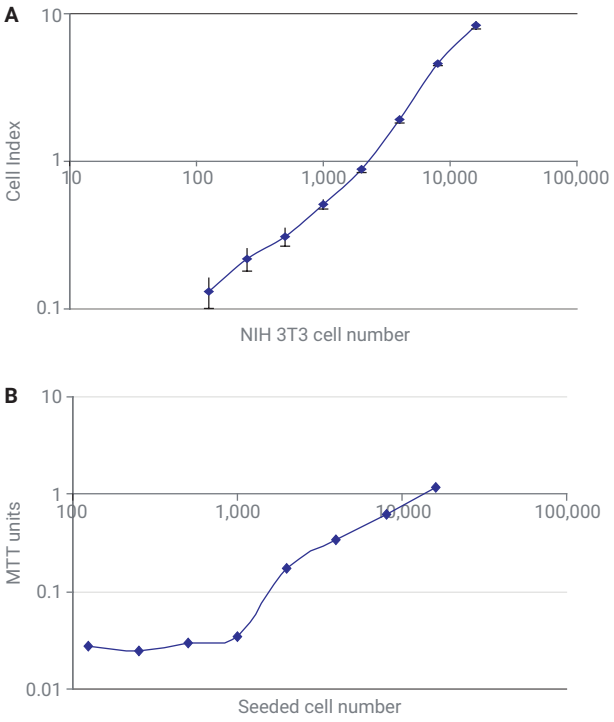

Figure 2. Correlation between Cell Index units and number of cells seeded and comparison with MTT. (A) Increasing numbers of NIH3T3, ranging from 100 cells to 10,000 cells, were seeded in 96X E-Plates. The cells were monitored for 10 hours, at which point the Cell Index was obtained. The Cell Index values were plotted against number of cells seeded. (B) At the end of the experiment described in Figure 2A, cells were assayed by MTT assay and the optical density at 590 nm was plotted against number of cells seeded.

Assessment of drug interaction with target cells

The IC_{50} value of tamoxifen was determined for different cell lines by impedance measurement to demonstrate that this new technology is suitable for measuring drug potency. These values were compared with MTT assay values measured 48 hours after tamoxifen was added. As shown in Table 1, the tamoxifen IC_{50} values obtained with the impedance-based system were consistent with values obtained by the MTT assay. This indicates that the system can be used to assess the potency of various drugs against different adherent cell lines.

To observe the kinetics of drug interaction with target cells, A549 nonsmall lung cancer cells were seeded in 96-well E-Plates and continuously monitored until the cells reached the log growth phase. Different concentrations of paclitaxel were then added to the cells. As shown in Figure 3A, the highest concentration of paclitaxel initially induced a cytotoxic effect, which is mainly due to cell death as determined by Annexin V staining (Figure 3B). Remarkably, the cells recovered from this initial cytotoxic effect and started to reproliferate.

This experiment shows the need for real-time measurement. The phenomenon observed in Figure 3A could have been missed if traditional single-point assays, such as MTT, had been used. In contrast, the impedance-based system uncovered this effect by showing the entire history of drug interaction with the target cells.

Table 1. Comparison of impedance technology versus MTT assay: IC_{50} values for tamoxifen treatment of different cancer cell lines. The indicated cell lines were either seeded in a standard 96-well microplate or seeded in 96X E-Plates and monitored with xCELLigence. Approximately 24 hours later, the cells were treated with increasing concentrations of tamoxifen and either continually monitored with the system or left incubating at 37 °C. After the experiment was stopped 48 hours later, the cells growing on standard 96-well microplates were assayed with MTT.

Cell Type	xCELLigence (µM)	MTT (µM)
HT1080	22.4	30.0
NIH3T3	16.0	19.0
HepG2	15.2	16.2
HUVEC	7.5	8.0

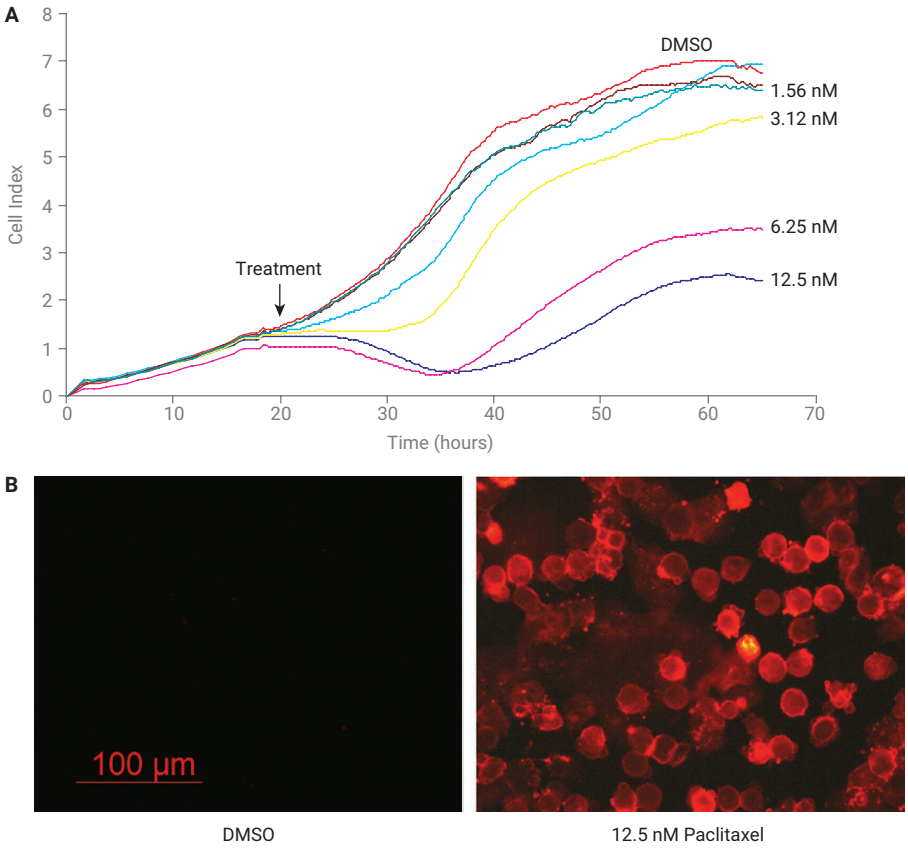

Figure 3. Dynamic monitoring of drug interaction with target cells. (A) A549 cells were seeded in 96X E-Plates at a density of 10,000 cells per well. The cells were continuously monitored up to 24 hours, at which point paclitaxel was added at various final concentrations. (B) Annexin V staining of A549 cells treated with DMSO or 12.5 nM paclitaxel for 20 hours. The cells were observed under a fluorescence microscope, and images were captured with an attached digital camera.

Dynamic monitoring of cell cycle arrest

Another major advantage of using the impedance-based system for continuous monitoring is that the user can gain insight into the mechanism of drug action. To demonstrate this, A549 cells were seeded into 96-well E-Plates and continually monitored by the system. The cells were treated with either DMSO (vehicle control) or with 100 µM olomoucine, a CDK inhibitor that induces cell cycle arrest either at the G1➔S transition or at the G2➔M transition, depending on the cell line.

As shown in Figure 4A, the addition of olomoucine to exponentially growing A549 cells caused the Cell Index to level off. It remained in a steady state that is reminiscent of cell cycle block, where the cells are not proliferating or dying. The control cells treated with DMSO continued to proliferate until they reach confluence, where they are contact-inhibited and the CI levels off a second time. To demonstrate that the effect of olomoucine on A549 cells was indeed due to an arrest of the cell cycle, A549 cells growing on the tissue culture dish were treated with the same concentrations of DMSO or olomoucine and subjected to flow cytometry analysis. As shown in Figure 4B, the flow cytometry analysis indicates that treatment of A549 cells with olomoucine induces cell cycle arrest at the G2➔M transition, where CDKs, such as CDK2, are active.

In summary, the new dynamic monitoring system affords the user an unparalleled opportunity to understand the mechanism of drug action.

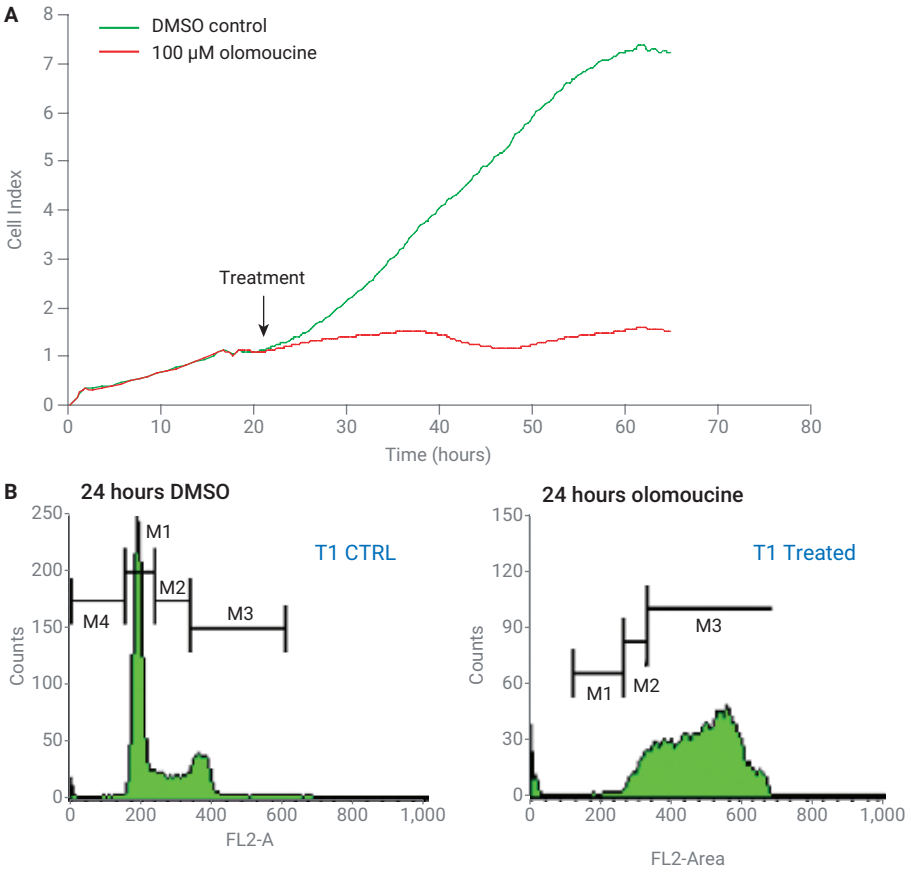

Figure 4. Dynamic monitoring of cell cycle arrest. (A) A549 cells were seeded on 96X E-Plates at 10,000 cells per well and continuously monitored using impedance technology. The cells were treated with either DMSO or 100 µM olomoucine. (B) A549 cells growing on tissue culture dishes for 20 hours were treated with DMSO or 100 µM olomoucine. Cell cycle analysis was performed using flow cytometry.

Dynamic monitoring of cytotoxic compounds

The impedance-based system was used to monitor the interaction of A549 cells with cytotoxic agents that have different mechanisms of action. This was done to demonstrate that the system is suitable for cytotoxicity analysis. Figure 5 shows the characteristic system traces obtained when A549 cells were treated with different concentrations of 5-fluorouracil, vinblastine, and staurosporine. The data show that each agent generates characteristic kinetic patterns depending on the cells used, the concentration of the drug, the duration of exposure, and the mechanism of drug action. Since each compound has its own characteristic pattern, these kinetic traces could potentially be used to determine the mechanism of action of compounds with unknown targets. The kinetic profile of the new compound could simply be compared with the profile of compounds that have known mechanisms of action.

In summary, impedance-based technology allows label-free and dynamic monitoring of cell proliferation, viability, and cytotoxicity. This system offers distinct and important advantages over traditional endpoint assays. It provides a built-in internal quality control to ensure consistency and reproducibility between the different assays. By recording the entire course of drug interaction with particular cells, dynamic monitoring allows users to gain a better understanding of the mode and mechanism of drug interaction. Finally, since each compound or drug has its own characteristic profile for its interaction with target cells, impedance-based technology may be useful for determining the mechanism of drug action with unknown targets.

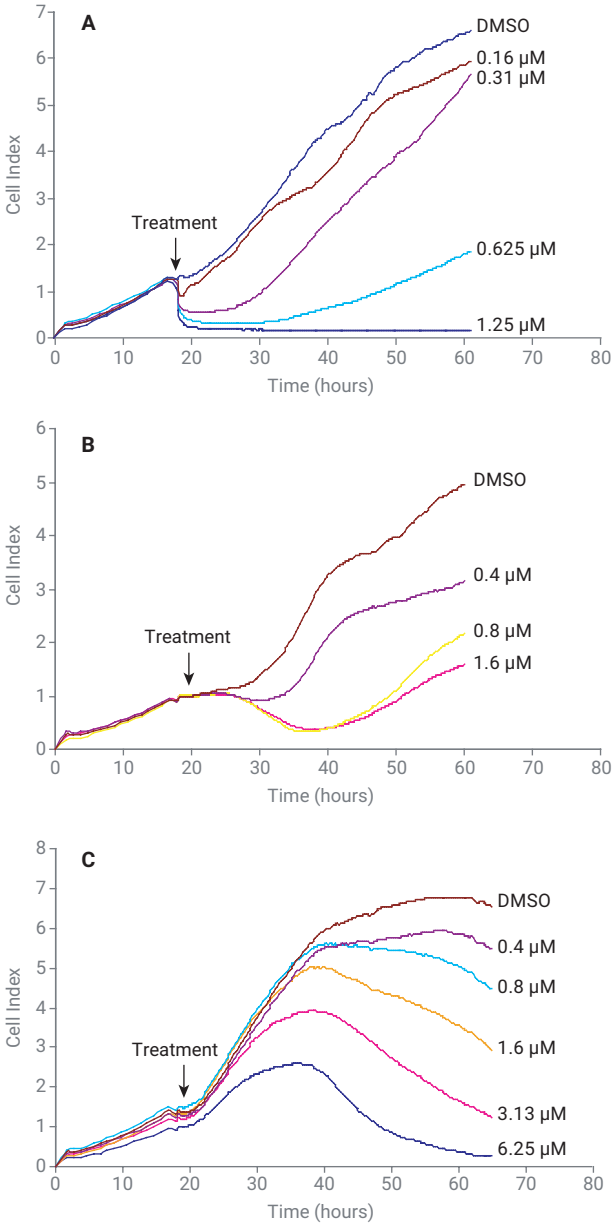

Figure 5. Dynamic monitoring of cytotoxic compounds with target cells. A549 cells were seeded in 96X E-Plates and continuously monitored using impedance technology. The cells were treated with the indicated final concentrations of (A) staurosporine, (B) vinblastine, and (C) 5-fluorouracil.

www.agilent.com/chem

For Research Use Only. Not for use in diagnostic procedures.

RA.0923958333

This information is subject to change without notice.

© Agilent Technologies, Inc. 2020
Printed in the USA, August 11, 2020
5994-1695EN

Agilent
Trusted Answers