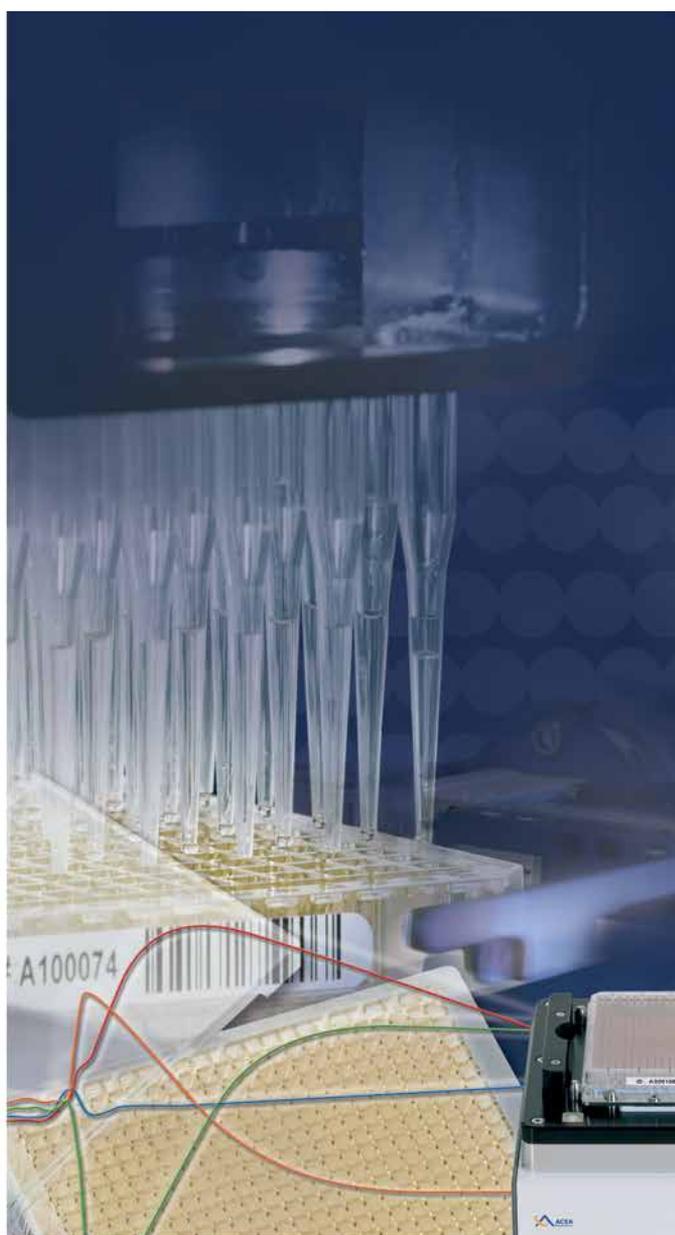


# xCELLigence System

Application Note No. 14 / January 2013

## xCELLigence System RTCA HT Instrument

Long-Term High-Throughput  
Cytotoxicity Profiling



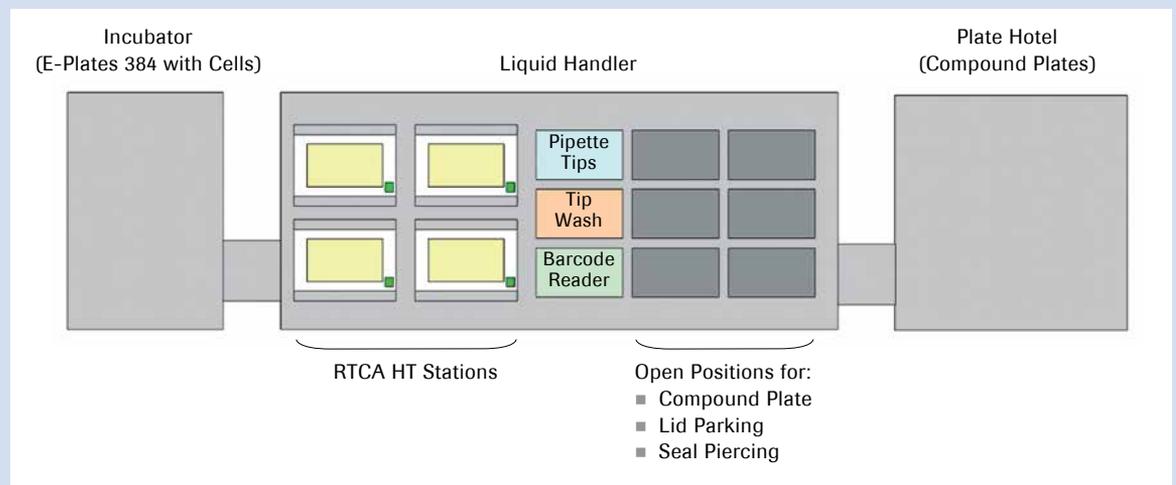
xCELLigence

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## Introduction

The current portfolio of xCELLigence System RTCA Instruments has brought label-free cell-based assays to the forefront of drug discovery research. The applications include cell proliferation, cytotoxicity, cell adhesion, cell invasion and migration, and receptor responses. These cover a wide range of disease areas including cancer, inflammation, diabetes, and infection. In addition to the label-free aspect of the RTCA Instruments, another main feature is real-time dynamic monitoring. Real-time monitoring of cellular processes provides important advantages over traditional endpoint assays. For the first time, a comprehensive representation of the entire time course of the assay is possible, allowing the user to make informed decisions regarding the timing of manipulations and treatments. Quantitative data of cellular kinetic responses provides important information about biological status, such as cell growth, arrest, morphological changes, and apoptosis. Precise time-dependent cell response profiles (TCRPs) provide important information regarding mechanism of drug action and off-target interactions underlying cytotoxicity (1).

The xCELLigence System RTCA HT Instrument makes all the features and benefits of the xCELLigence System technology available to high-throughput compound screening and profiling. The RTCA HT Instrument consists of the RTCA HT Control Unit with the RTCA HT Software, the RTCA HT Analyzer, and up to four RTCA HT Stations. Each HT Station holds a 384-well, Society for Bio-molecular Sciences (SBS)-compatible plate (E-Plate 384) for measuring cell responsiveness to agents using the principle of electrical impedance. Cells are seeded one day prior to treatment for attachment to the electrodes located in the bottom of the E-Plate 384 wells. After the E-Plate 384 is placed on the RTCA HT Station, cellular interactions are detected by microelectrodes of the RTCA HT Analyzer. Changes in electrical impedance are produced after a small current is run through the microelectrode sensors in the bottom of each well of the E-Plate 384. Impedance values are converted to Cell Index (CI) values by the RTCA HT Software. CI values reflect the number of cells, as well as morphological parameters, such as size, shape, and



**Figure 1: Schematic showing the RTCA HT Instrument incorporated into a typical automation platform configuration.** Up to four RTCA HT Stations may be incorporated directly onto the deck of the automated liquid handling platform; these are connected by cables to the adjacent RTCA HT Analyzer (not shown). In a typical assay, the E-Plate 384 containing growth media (dispensed on- or off-line) are loaded into the incubator and background impedance readings are taken on the RTCA HT Station after barcode reading. Cells are then plated onto the E-Plates 384 (dispensed on- or off-line), which are again

loaded into the incubator. The E-Plate 384 may be monitored for cell growth over time by cycling in and out of the RTCA HT Stations at regular intervals, where up to four plates may be read simultaneously for a user-defined number of independent impedance readings. For compound addition, the user's standard automation workflow may be utilized. Compounds may be added to the E-Plate 384 at an open position or, in cases where detection of rapid response kinetics is required, directly on the RTCA HT Station while the cells are being monitored for impedance changes.

degree of cell attachment to the substrate. Sampling time can be set as short as 15 seconds for real-time readout of rapid responses, such as those mediated by GPCR activation and long-term cytotoxic effects. A heating unit in the RTCA HT Station ensures that all assays are performed at physiological temperature, ensuring biological relevance under screening conditions. The new RTCA HT Instrument platform is compact for integration into existing liquid handling and automation workflows (see Figure 1).

In this application note, we show that different cell lines exhibit distinct profiles using the RTCA HT Instrument. These findings underscore that this system is useful for high-throughput screening of cell phenotype that alters its cell attachment, morphology, and growth characteristics. In addition, we show that antiproliferative agents, through different mechanisms of action, exhibit distinctive kinetic profiles using the RTCA HT Instrument.

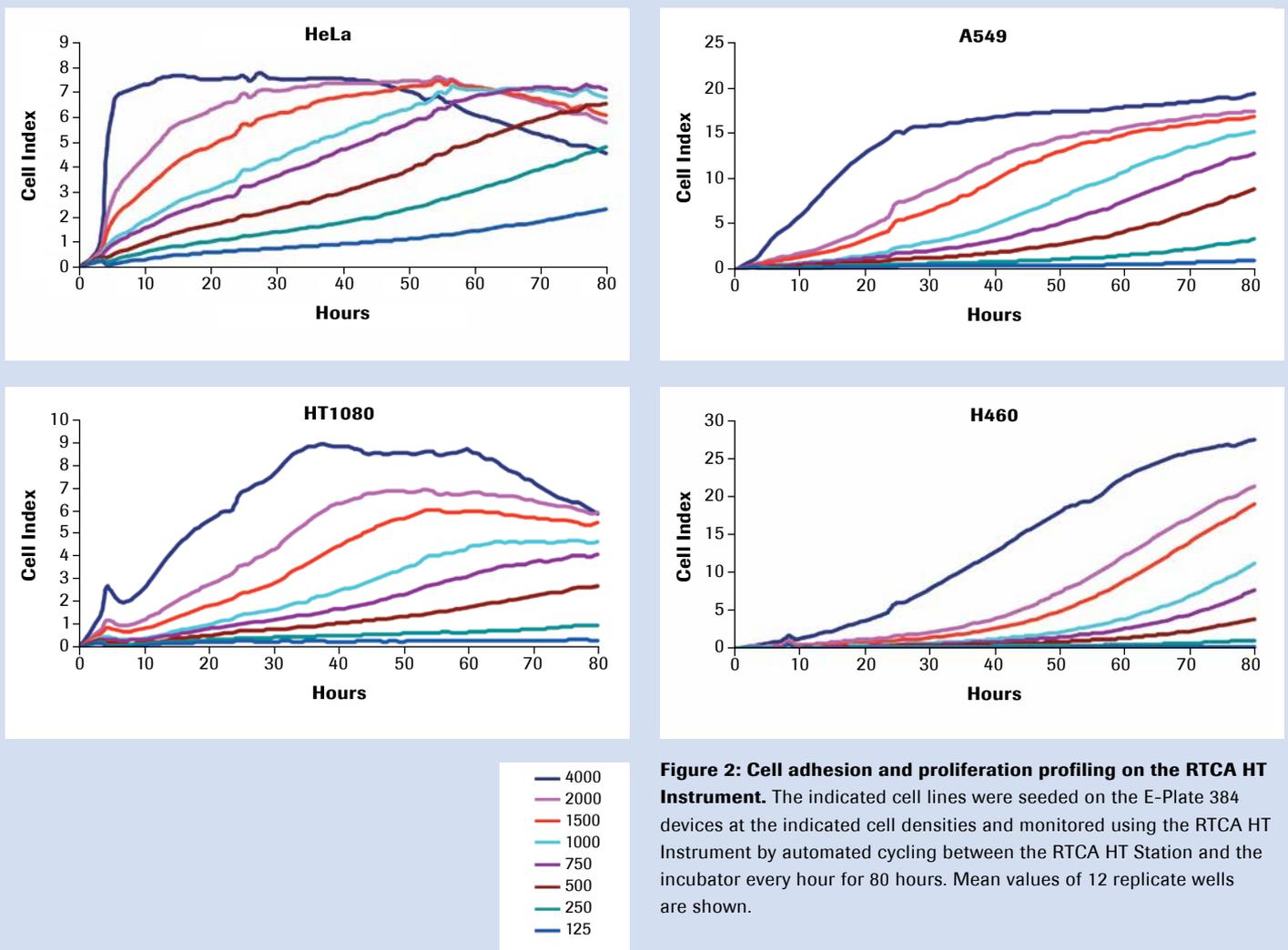
In addition, compound potencies measured on the RTCA HT Instrument are correlated with those obtained in standard endpoint assays. Comparing the kinetic profile of one of these compounds to the kinetics of apoptosis induction revealed a high correlation between the two assays, illustrating the applicability of kinetic readout for pinpointing the optimal time points for performing endpoint assays. In summary, the new RTCA HT Instrument proved to be user-friendly for developing assays that screen long-term cellular responses in high-throughput workflows using 384-well culture plates.

## Results

### Dynamic Monitoring of Cell Proliferation on the RTCA HT Instrument

To characterize growth profiles of different cell lines using the RTCA HT Instrument, four human cell lines were chosen: the HeLa cervical cancer cell line, A549 and H460 lung carcinoma cell lines, and the HT1080 fibrosarcoma cell line. Different numbers of cells (125 to 4,000 per well) were seeded and growth was monitored every hour for 80 hours (see Figure 2). Each cell line produced distinct profiles with the RTCA HT Instrument, corresponding to differences in growth rate, cell morphology, and attachment quality. For example, at a density of 2,000 cells per well, HT1080 and HeLa cells reached the maximal

Cell Index value at about 30 hours, while A549 and H460 cells reached maximum at 80 to 90 hours. Each cell line exhibited a unique kinetic profile, dependent on cell size and degree of attachment to the surface of the plate. These characteristic profiles may be used to verify homogeneity and monitor for contamination of each cell line, as well as for accurate calculation of attachment kinetics, doubling time, and different growth phases (2). Such parameters may be of interest in screens for modulators of differentiation or other long-term cellular responses, which may be conducted over a span of days (or even weeks) using the integrated RTCA HT Instrument.



### Dynamic Monitoring of Antiproliferative Agents on the RTCA HT Instrument

xCELLigence System technology has proven to be useful for screening for antiproliferative agents. The kinetic profiles generated can indicate a mechanism of action and identify potential off-target or toxic effects (1). To investigate the utility of the RTCA HT Instrument in assessing the effects of antiproliferative agents on target cells, four well-characterized compounds were tested: two DNA-damaging agents (camptothecin and doxorubicin), a proteasome inhibitor (MG132), and a PI3K kinase/mTOR inhibitor (PI-103). A549 lung carcinoma cells were seeded on E-Plates 384, grown overnight, and treated with the indicated concentrations of compound while still in logarithmic growth phase, and cell responses monitored by taking xCELLigence System readings every hour for 72 hours (see Figure 3). All compounds mediated a decrease in Cell Index, as expected for any antiproliferative agent; however, the kinetic profile of the cellular response differed in a dose-dependent manner with each compound's mechanism of action. The DNA-damaging agents generated a transient increase in Cell Index immediately after compound addition, possibly reflecting a transient morphological change, followed by a gradual, dose-dependent decrease in Cell Index (see Figure 3A, B; left panels). The proteasome inhibitor MG-132 produced a similar profile with slightly more rapid onset (see Figure 3C; left panel). In contrast, the PI3 kinase/mTOR inhibitor PI-103 produced a more gradual decrease in Cell Index over the entire course of the experiment (see Figure 3D; left panel).

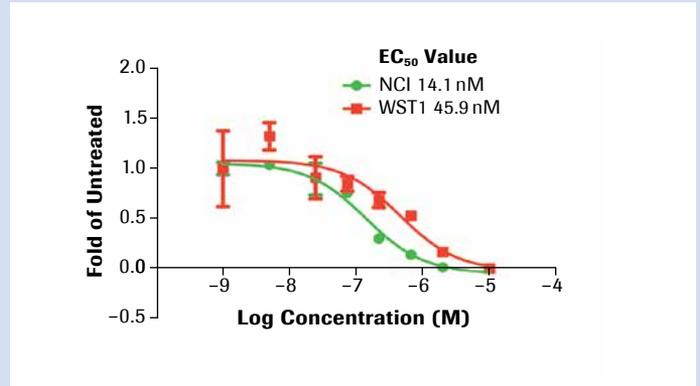
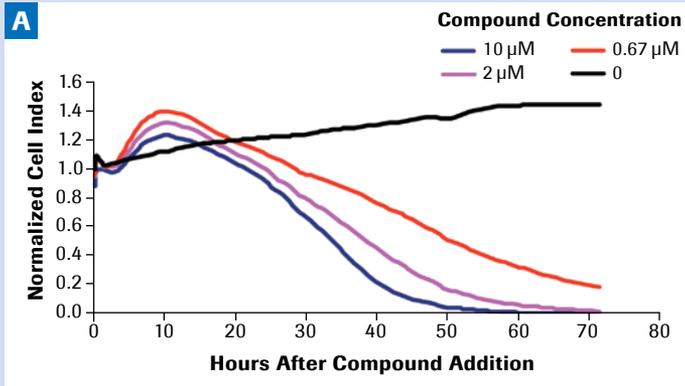
To verify that the Cell Index values measured by the RTCA HT Instrument correlate with the antiproliferative effects of these compounds, an independent, colorimetric-based cell proliferation assay (WST-1) was performed in a parallel experiment. A549 cells were cultured in an E-Plate 384 and treated as above. At 72 hours after compound addition, the WST-1 reagent was added, the plate was incubated for 1 hour, and the lysate was transferred to a regular 384-well plate for absorbance measurements.

The resulting dose-response curves and calculated  $EC_{50}$  values obtained using both the RTCA HT Instrument and the WST-1 assay are shown for each compound in Figure 3 (A-D; right panels). These curves were very similar between the WST-1 and xCELLigence System assays, indicating that quantitative changes in Cell Index accurately reflect changes in cellular viability.

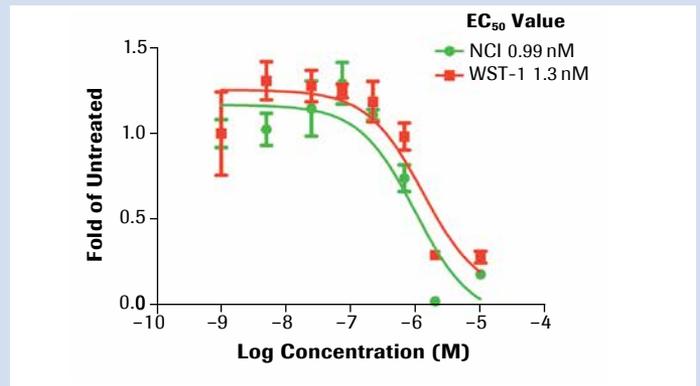
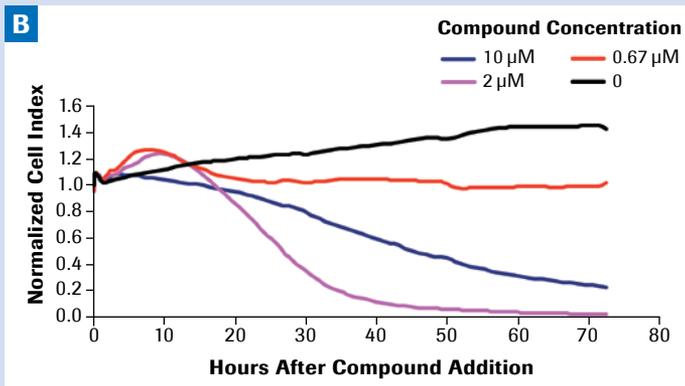
The DNA-damaging agents and the proteasome inhibitor tested here ultimately induce apoptosis in A549 cells, albeit with different kinetics. To investigate the relationship between the kinetic Cell Index response profile generated using the RTCA HT Instrument and the kinetics of apoptosis induction, the Cell Death Detection ELISA Kit was used. A549 cells were treated with the DNA-damaging agent camptothecin, as described above and monitored using the xCELLigence System. Identical experiments were conducted in parallel using E-Plate 96, and subjected to the Cell Death Detection assay at 24, 48, or 72 hours after compound addition.

Representative results for Cell Index monitoring and the apoptosis assay of one dose of camptothecin are shown in Figure 4 (A and B). Robust apoptosis induction was observed at the 24 and 48 hour time points (see Figure 4B), corresponding to a time shortly after the Cell Index value begins a rapid decrease (approximately 18 hours), and the time at which it reaches the minimal value, respectively (approximately 50 hours; see Figure 4A). At 72 hours, the apoptotic signal is greatly reduced, suggesting that most of the cells are dead. At the 48 hour time point, the dose-response profiles in the two assays are nearly perfect mirror images; as apoptosis induction is detected with increasing camptothecin concentration, the Cell Index value is correspondingly decreased (see Figure 4C). In addition, the calculated  $EC_{50}$  values are comparable in the two assays (see Figure 4C). Clearly, the RTCA HT Instrument provided a quantitatively accurate measure of apoptosis induction. Taken together, these findings illustrate the utility of the real-time kinetic readout for determining the optimal time points for conducting follow-up assays.

Camptothecin

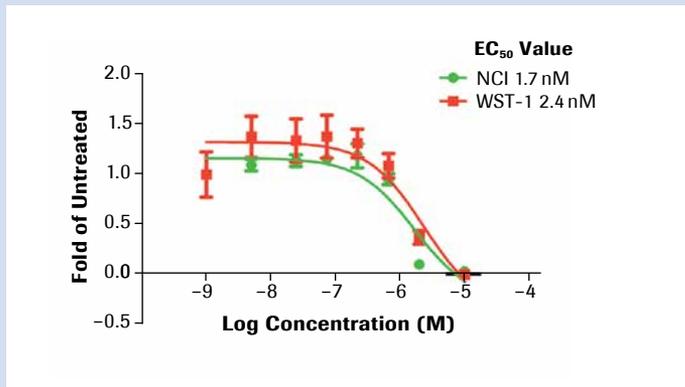
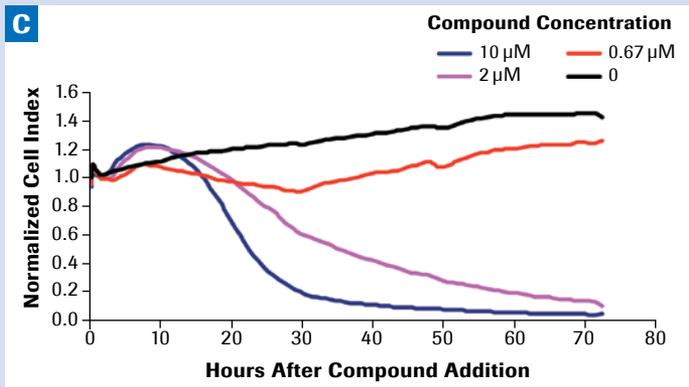


Doxorubicin

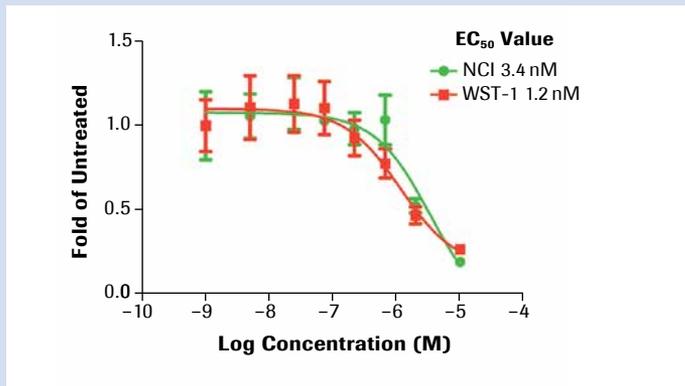
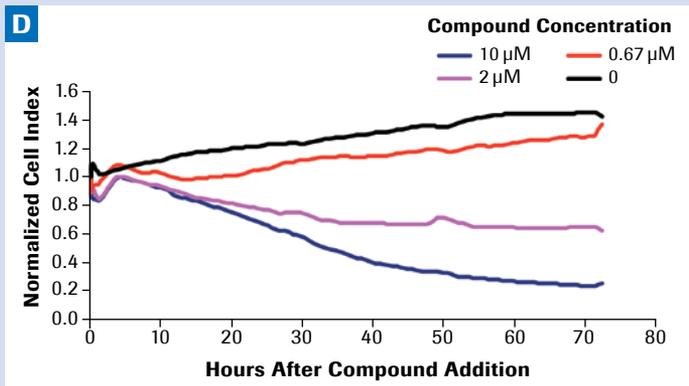


**Figure 3: Profiling of cytotoxic agents on the RTCA HT Instrument.** A549 lung carcinoma cells were seeded on the E-Plate 384 devices at 4,000 cells per well and grown overnight. The indicated compounds were added at indicated concentrations. Responses were monitored hourly by automated cycling between the RTCA HT Station and the incubator. Left panels show cellular responses measured using the RTCA HT Instrument; Normalized Cell Index (NCI) values are plotted as the mean value

MG-132

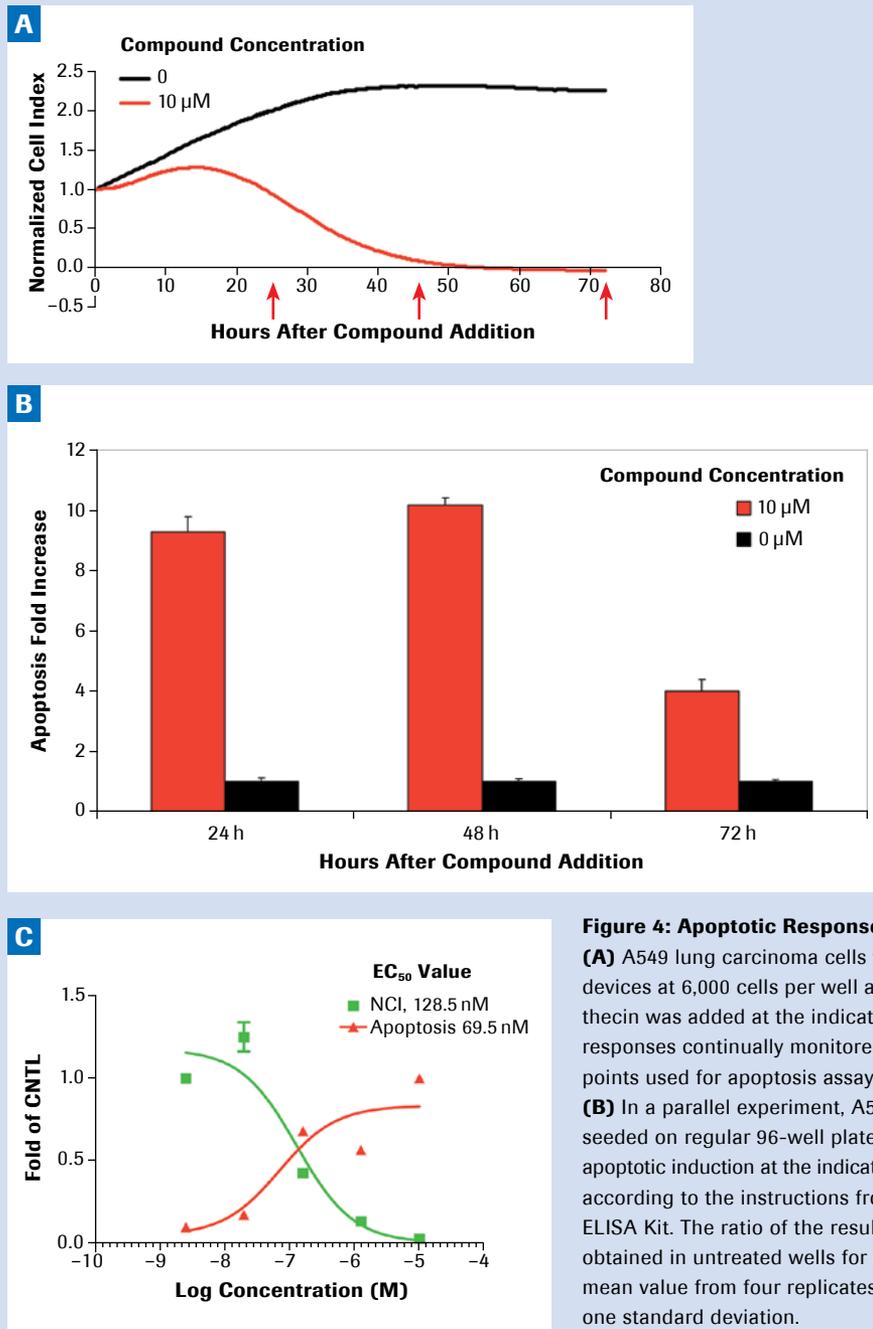


PI-103



from four replicates, with error bars indicating one standard deviation. Only selected concentrations are shown for clarity. Right panels show the dose-response curves from the 72 hour time point from both the xCELLigence System and from WST-1 assays performed in parallel experiments. Values are plotted as the mean value from four replicates, with error bars representing one standard deviation. The EC<sub>50</sub> values derived from each assay are shown.

Camptothecin



**Figure 4: Apoptotic Response Comparison.**

**(A)** A549 lung carcinoma cells were seeded on the E-Plate 96 devices at 6,000 cells per well and grown overnight. Camptothecin was added at the indicated concentrations and cellular responses continually monitored. Red arrows indicate time points used for apoptosis assays.

**(B)** In a parallel experiment, A549 lung carcinoma cells were seeded on regular 96-well plates, treated as in **(A)**, and apoptotic induction at the indicated time points was determined, according to the instructions from the Cell Death Detection ELISA Kit. The ratio of the resulting apoptosis signal to that obtained in untreated wells for each dose is plotted as the mean value from four replicates, with error bars representing one standard deviation.

**(C)** Dose-response curves derived from data obtained at the 48 hour time point in **(A)** and **(B)**. Cell Index values were normalized (NCI) as in **(B)**, and the EC<sub>50</sub> values derived from each assay are indicated. CNTL = untreated samples.

The RTCA HT Instrument is ideal for screening compounds that elicit long-term cellular responses. The integration of up to four RTCA HT Stations with an automated plate-handling system permits the continual cycling of a large number of plates between incubator and station. This feature, combined with a rapid plate-reading time (as little as 15 seconds), produces a high-throughput rate. The RTCA HT Station's built-in heater ensures that all readings obtained are at physiological temperature, minimizing disruption of cellular processes during readings and over the course of the screen. In this way, biological responses occurring over a time scale of days or even weeks may be reliably used for small molecule screening by the RTCA HT Instrument.

Our findings show that cells with distinctive morphology, attachment, and growth characteristics produce biologically meaningful profiles when assayed by the RTCA HT Instrument (see Figure 2). Disease-relevant changes in these cellular characteristics, such as cell death to more subtle cell morphological changes, such as those resulting from differentiation processes, may be effectively screened using the RTCA HT Instrument. For the antiproliferative agent tests conducted here, the RTCA HT Instrument exhibited high sensitivity, with calculated  $EC_{50}$  values obtained in high correlation with the WST-1 and apoptosis assays. The major advantage of the xCELLigence System assay compared to standard assays stems from the kinetic nature of the readout. Previous work has established that the time-dependent cell response profile (TCRP) obtained from small molecule or siRNA treatments using the xCELLigence System may be indicative of the mechanism of action, including the identification of undesirable off-target effects (1, 3). Indeed, for the four antiproliferative agents tested here, three distinct mechanisms of action were involved, as indicated by the different TCRP profiles.

These kinetic patterns can also be used to determine the mechanism of action of novel compounds interacting with unknown targets by comparison to compounds with known mechanisms. In this case, the TCRP profiles can be seen as a reflection of the inherent global cellular response to a specific treatment, all detectable as measurable effects on cell adhesion, morphology, and proliferation, which may be revealed in the primary screen, including undesirable and cytotoxic effects. The RTCA HT Instrument expands the biological space and the time resolution in which a compound is screened, providing unique profiles that can be used in a high-throughput manner to categorize compounds with important biological activities based on their mechanism of action, all from primary screen data.

### Summarizing:

- The RTCA HT Instrument is well-suited for high-throughput screening of cellular responses over long experimental time periods.
- Cells with different attachment, morphologies, and growth rates produce distinct kinetic profiles obtained on the RTCA HT Instrument.
- Antiproliferative agents produce distinct profiles on the RTCA HT Instrument, corresponding to their different mechanisms of action.
- Compound potencies as measured using the RTCA HT Instrument are correlated with widely used and more time-consuming functional endpoint assays.

## References

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## Ordering Information

Product	Cat. No.	Pack Size
<b>RTCA HT System Bundle</b>	<b>00380601070</b>	<b>1 Bundle Package</b>
<b>RTCA HT Station</b>	05919878001	1 Instrument
<b>RTCA HT Analyzer</b>	05919843001	1 Instrument
<b>RTCA HT Control Unit</b>	06200176001	1 Instrument
<b>E-Plate 384</b>	05867681001	2 × 5 Plates
	05867673001	8 × 5 Plates

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The RTCA HT Analyzer in combination with the RTCA HT Station and an RTCA HT Software Package 1.x, with the E-Plate 384, is a real-time cell based assay system covered by US patent No. 7,192,752 (exp. 11/10/2023), No. 7,470,533 (exp. 1/12/2025), No. 7,560,269 (exp. 10/24/2025).

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