High Throughput Multiplexed Apoptosis Assays Using the Labcyte Echo® Liquid Handler and the IntelliCyt iQue® Screener HD

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Abstract

The need for characterizing apoptotic processes occurs throughout the drug discovery process – from primary screening to toxicity profiling. Apoptosis is a tightly regulated cell death program that can be executed by cells that are no longer physiologically necessary. It is often triggered as a response to extrinsic factors or inhibited for survival as in the case of many cancer cells. Cell death cascades are complex and dynamic, underscoring the importance of a multi-parametric approach to assess apoptosis. This underscores the need to conduct robust and reliable cellular assays at higher densities and with smaller sample sizes. As such, technological advancements such as High Throughput Flow and low-volume liquid handling have become critical components of methods assessing apoptosis. Using the IntelliCyt iQue Screener HD and MultiCyt® 4-Plex Apoptosis Screening Kit in conjunction with the Labcyte Echo liquid handler, we were able to simultaneously detect 4 different apoptosis endpoints in Jurkat cells in both 384- and 1536-well formats.

In this study, Jurkat cells were treated for 24 hours with known apoptosis inducing agents: staurosporine, nocodazole and camptothecin. After treatment, cells were labelled for one hour with a no-wash / single step addition of fluorescent markers for caspase 3/7 activation, Annexin V binding, cell viability, and mitochondrial membrane depolarization. Sub-microliter volumes of compounds and dye were transferred to the 384- and 1536-well cell plates with the Echo liquid handler. The results show equivalent potency estimates for the compounds tested in both plate formats and correlate to previously reported activity for the biomarkers measured.

IntelliCyt iQue Screener HD

Figure 1: IntelliCyt iQue Screener HD. The iQue systems incorporate IntelliCyt's high throughput flow technology, which extends the capabilities of flow cytometry by dramatically increasing throughput and decreasing sample size. A patented sampling method transfers assay components in suspension (including cells, beads, yeast and bacteria) to the detector in a continuous, air gap delimited stream. Up to 60,000 data points per second are collected, including four fluorescence channels and two label-free channels.

Methods

The non-adherent human T-cell line (Jurkat, cloneE6.1 ATCC) was maintained at log growth phase in RPMI 1640 supplemented with 10% cosmic calf serum and 2X L-glutamine under standard conditions (37°C, 5% CO2). For apoptotic induction, staurosporine, nocodazole and camptothecin (Sigma-Aldrich) were diluted to the appropriate concentration in DMSO. Ten- point dose response plates were prepared by 1:3 serial dilutions in duplicate to create assay ready screening plates with the ECHO 555 liquid handler at 200x FAC (0.5%DMSO); 100 nl for 384 and 25 nl for 1536 assay plates. Cells at 1E6/ml were added with a Thermo Scientific MultiDrop® Combi reagent dispenser, 20µl for 384 and 5µl for 1536, and incubated under standard conditions with appropriate plate controls. After 24 hour incubation, the dye cocktail was added in a single addition of 1.14 µl for 384 and 285 nl for 1536 with the Echo 525 liquid handler. After one-hour incubation at room temperature, data was acquired using the iQue Screener HD. Data was processed using the ForeCyt® Control and Analysis Software and dose response analysis of data was fit with a four parameter logistic function using GraphPad Prism.

Apoptotic markers measured by the MultiCyt 4-Plex Apoptosis Screening Kit

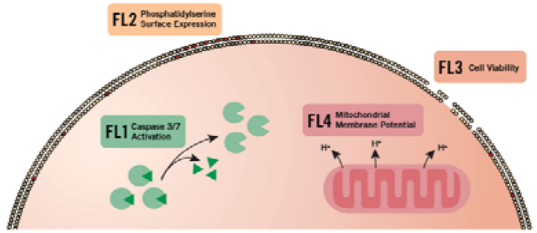

Figure 2: The MultiCyt Apoptosis Screening kit simultaneously measures 4 apoptosis related markers: 1) Caspase 3/7activation, 2) Phosphatidylserine surface expression; 3) Cell viability, 4) Mitochondrial membrane potential. Cell numbers are also determined for each sample.

Results: 384 Well Assay

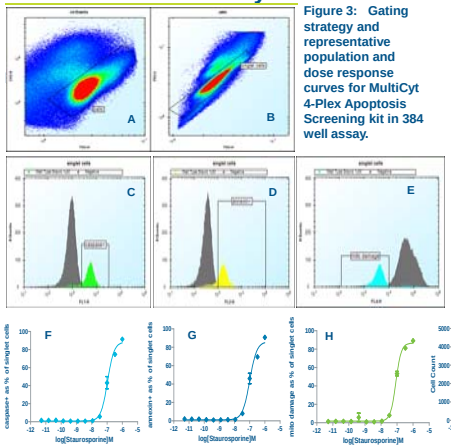

Figure 3: Gating strategy and representative population and dose response curves for MultiCyt 4-Plex Apoptosis Screening kit in 384 well assay.

Figure 3(A-I):
3A)Gating All Cells
3B)Gating Singlet Cells
3C) Caspase + Cells
3D) Annexin + Cells
3E) Mitochondrial Damage Cells
3F) Staurosporine Induced Caspase + Dose Response
3G) Staurosporine Induced Annexin + Dose Response
3H) Staurosporine Induced Mitochondrial Damage Dose Response
3I) Staurosporine Dose Response and Cell Count

Results: 1536 Well Assay

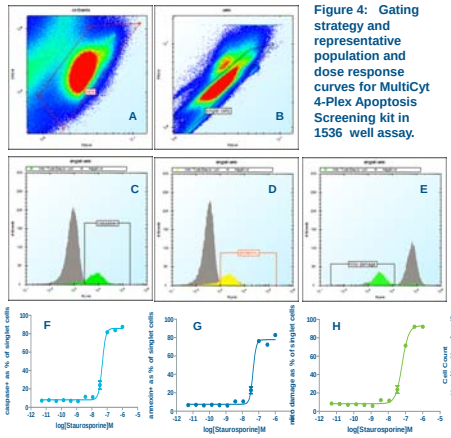

Figure 4: Gating strategy and representative population and dose response curves for MultiCyt 4-Plex Apoptosis Screening kit in 1536 well assay.

Figure 4(A-I):
4A)Gating All Cells
4B)Gating Singlet Cells
4C) Caspase + Cells
4D) Annexin + Cells
4E) Mitochondrial Damage Cells
4F) Staurosporine Induced Caspase + Dose Response
4G) Staurosporine Induced Annexin + Dose Response
4H) Staurosporine Induced Mitochondrial Damage Dose Response
4I) Staurosporine Dose Response and Cell Count

The Echo 500 Series Liquid Handler

Figure 5: The Echo 555 Liquid Handler. The Labcyte Echo 500 series revolutionizes liquid transfer by using acoustic energy to eject fluids. The Echo liquid handler is completely touchless—no tips or nozzles, and no material contacts the sample as it moves from source to destination. The elimination of tips when using the Echo liquid handler provides additional cost savings and eliminates waste, carry-over effects, and cross-contamination. Additionally, dispensing sub-microliter volumes of reagents saves time and reagent. The Echo 550 and 555 liquid handlers transfer in 2.5 nL droplet increments, and the Echo 525 liquid handler transfers in 25 nL droplet increments.

Experiment Summary

Caspase 3/7 Activation

	384	1536	1536 n2	Average	StDev	nM Avg
Staurosporine	1.07E-07	3.23E-08	4.74E-08	6.24E-08	3.96E-08	62.36
Nocodazole	3.61E-08	3.07E-08	3.67E-08	3.07E-08	3.31E-09	30.68
Camptothecin	1.37E-08	1.22E-08	1.05E-08	1.22E-08	1.60E-09	12.16

Annexin V Binding Phosphatidyl Serine

	384	1536	1536 n2	Average	StDev	nM Avg
Staurosporine	1.00E-07	3.28E-08	4.41E-08	7.22E-08	3.97E-08	72.22
Nocodazole	3.53E-08	3.17E-08	3.63E-08	3.44E-08	2.39E-09	34.43
Camptothecin	1.37E-08	1.23E-08	1.05E-08	1.22E-08	1.60E-09	12.15

Mitochondrial Membrane Potential

	384	1536	1536 n2	Average	StDev	nM Avg
Staurosporine	8.49E-08	2.54E-08	6.29E-08	5.77E-08	3.01E-08	57.71
Nocodazole	3.64E-08	3.02E-08	3.67E-08	3.44E-08	3.67E-09	34.44
Camptothecin	1.48E-08	1.05E-08	1.09E-08	1.21E-08	2.37E-09	12.06

Cell Count

	384	1536	1536 n2	Average	StDev	nM Avg
Staurosporine	3.27E-08	3.18E-08	3.55E-08	3.33E-08	1.92E-09	33.33
Nocodazole	3.55E-08	3.29E-08	3.34E-08	3.44E-08	1.43E-09	34.44
Camptothecin	1.20E-08	8.43E-09	8.78E-09	9.72E-09	1.94E-09	9.72

Summary

- The MultiCyt Apoptosis Screening kit was validated using the Echo liquid handler and the iQue Screener HD.
- The Echo enabled the total assay volume in 384 well to be reduced from 40 µl to 21.24 µl resulting in time and reagent cost reduction. The assay was miniaturized in 1536 to less than 6µls further increasing throughput.
- Compound dosing and dye additions with the Echo liquid handler produced equivalent results in both the 384 well and 1536 well assay that correlated with IntelliCyt published values.
- The miniaturization of assay volumes and validation at higher density with the Echo liquid handler series maximizes the potential of High Throughput Flow with the iQue Screener HD.

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