

Automating High Content Screening Assays

Joseph Zock, Megan Weiss, Alan Stoumen, and Amy Drzal

Abstract

The development of the High Content Screening (HCS) platform using the ArrayScan HCS System allows a new generation of multi-parameter, cell based assays to be utilized in the early drug discovery process. It is important for HCS reagents and protocols to be compatible with liquid handling and microplate preparation automation typically found in the pharmaceutical lab environment. In conjunction with our corporate partner, Beckman Coulter, Inc., we have adapted our HiKit™ HCS Reagent Kits to several automation platforms including the Biomek® 2000. Additionally we have evaluated and currently use several cell washers including the MAP-C (Titertek; Huntsville, AL) and the EL405 (Bio-Tek; Winooski, VT). Examples of automated HCS will be presented along with performance and validation data.

Introduction

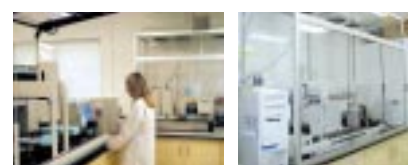
Compound screening for biological activity is the cornerstone of the drug discovery process and has become an important part of all phases including target identification, target validation, and lead optimization. When screening one must consider three basic assay parameters: biological relevance, robustness, and capacity. The proper balance of these issues has historically been a compromise between biological relevance achievable and the screening capacity required. Capacity and robustness are impacted by the ability to apply automation tools to the assays being considered. Celomics, Inc. is addressing all three issues with the development of the HCS platform for cell based assays. The HCS assays developed to date, and those on the horizon, provide an unprecedented ability to measure biologically relevant targets, from tracking multiple signaling molecules in the same cell to quantifying complex phenotypes like morphology changes or cell motility. Developing the ArrayScan HCS platform to automate the detection, quantification, and visualization of cellular events in standard microplates was a significant leap forward in both the robustness of the measurements, and the capacity to screen. However, capacity of the detector is only one part of the screening equation and consideration must be given to assay preparation including cell plating, compound addition, incubation, and post assay processing.

The automation of assay preparation for screening typically includes the use of many different types of liquid handling robotics. Many commercially available systems are currently in use by the pharmaceutical industry in all facets of the drug discovery process. To establish that HCS is compatible with these systems, Celomics assembled a "pharma emulation" laboratory where all basic liquid handling functionalities are represented. Within this environment we have been able to evaluate different peripheral automation instruments and use combinations of them to semi-automate the majority of our HiKit assays. Herein we report several examples of both automated assay validation and small scale "screening" using combinations of peripheral automation. Most assay procedures used in this report are taken directly from protocols included with the HiKit products.

Celomics is a Registered Trademark of Celomics, Inc. Copyright © 2001 Celomics, Inc. All Rights Reserved.

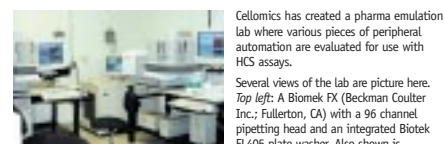
Email: info@celomics.com
www.celomics.com

Figure 1: Examples of Peripheral Automation Used to Automate HCS Plate Preparation



Biomek FX and MAP-C

Biomek 2000



ArrayScan HCS System

filtration (AirFiltrix Inc.; Clifton, NJ) Top right: A Biomek 2000 with a standard wash set including an eight channel wash head, also hooded. Bottom: The ArrayScan HCS Systems and Celomics® Store data warehouse that support our group.

This table describes equipment we are currently using to automate the production of HCS assay plates. It is not meant to be an inclusive list, but rather shows that HCS assay protocol steps can be automated using conventional instruments and techniques.

Automation	Vendor	Use in HCS
Multidrop® 96/384	Titertek Huntsville, AL	<ul style="list-style-type: none"> Cell Plating Bulk additions Media replacement
MAP-C	Titertek Huntsville, AL	<ul style="list-style-type: none"> Bulk addition of dyes and stains Fixative Permeabilization Solution Antibodies Buffers Washing cell layer Multiple step protocols and incubations
EL405	Biotek Winooski, VT	<ul style="list-style-type: none"> Washing cell layer
Biomek® 2000	Beckman Coulter Fullerton, CA	<ul style="list-style-type: none"> Additions to control wells Serial dilution Individual well cherry picking Multiple step protocols and incubations
Biomek® FX	Beckman Coulter Fullerton, CA	<ul style="list-style-type: none"> Whole plate addition Assay plate replication Compound library distribution Multiple step protocols and incubations
CyberLab C250	Gilson Middleton, WI	<ul style="list-style-type: none"> Whole plate addition Assay plate replication Compound library distribution Multiple step protocols and incubations

Figure 2: Automation of HCS Protocols Can Reduce Variability

Comparing the coefficient of variation (COV) for individual wells (n=3) between plates either prepared manually or using a combination of automation for the liquid handling and plate washing steps in a prototype cytotoxicity assay protocol. COVs are plotted for two output features in this assay, nuclear area and mitochondrial mass. Data for these measurements were collected simultaneously on the cells in the wells of microplates in triplicate. Although automated plate preparation results in reduced COVs per well it is interesting to note that individual output feature measurements made in multi-parameter HCS assays will have independent overall variability, depending on the biology involved.

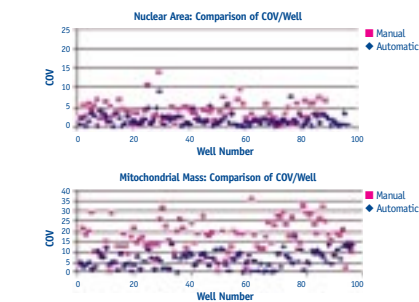


Figure 3: Automation Generates Valid HCS Data

The NFAT-1 activation HiKit product was used to test compatibility of the protocol and reagents with peripheral automation. The assay, based on immunocytochemistry, tags endogenous transcription factor NFAT-1 and tracks activation by accumulation in the nucleus of cells. Several instruments were used to automate the assay preparation including the Multidrop, Biomek2000, Cyberlab C-250, and MAP-C. A standard validation run of maximum signal plates (2), minimum signal plate (2), and a dose response curve plate was completed for three consecutive days and the resulting data were analyzed for overall variability and any anomalies.

The summary results for the validation in HeLa cells are shown with the maximum signal COVs between wells, plates, and days highlighted. These results are consistent with validation data generated manually and produced lower COVs overall.

Summary of Automated Validation of NFAT-1 Assay				
Well-to-Well Variation:	Mean of COVs	Stand Dev		
Max Plates:	10.51	0.96		
Min Plates:	21.20	2.32		
Plate-to-Plate Variation:	Means of Means	Stand Dev	COV (sdev)	
Max Plates:	91.04	5.31	5.83	
Min Plates:	13.14	2.29	17.47	
Day-to-Day Variation:	Day 1	Day 2	Day 3	
Max Plates:	Mean 91.46	SD 4.86	COV 5.32	93.87
Min Plates:	Mean 15.20	SD 0.29	COV 1.93	13.54
	Mean 2.66	SD 0.36	COV 2.66	14.14
	Mean 1.20	SD 0.12	COV 1.20	8.51
Variation Between Days:	Mean of Means	Stand Dev	COV	
Max Plates:	91.04	5.14	5.65	
Min Plates:	13.14	2.42	18.43	

An example of a maximum signal plate evaluated for anomalies. No edge effect trends are apparent in the plates prepared by automated methods. The Y axis ("Value") is the cytoplasmic to nucleus difference of the fluorescent intensity of labeled NFAT-1.

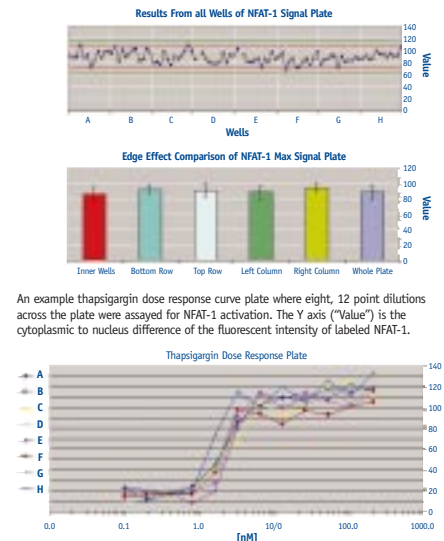


Figure 4: Screening a "Toxin" Library with Automated HCS Assays

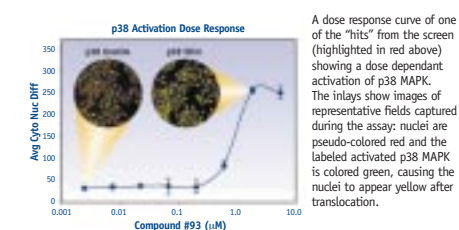
A commercially available library of 160 known toxic "killer" compounds (Microsource Discovery Systems; Gaylordville, CT) were screened for p38 MAPK activation and for cytotoxic activity in a prototypic Multiparameter Cytotoxicity-1 Assay. Both of these assays were automated using a variety of instruments (see below). In order to assess variability of multiple parameters and ability to see incremental changes the assays were run as dose response curves, eight compounds to a plate, run in triplicate.

A table showing the instruments applied to various protocol steps in the two assays used.

Peripheral Automation	P38 MAPK Protocol Steps	MP Cytotox Protocol Steps
Multidrop	Adding cells to assay plates	Adding cells to assay plates
Biomek 2000	Compound reformatting Serial dilutions of compounds	Compound reformatting Serial dilutions of compounds
Cyberlab C250	Compound transfer to assay plates	Compound transfer to assay plates Addition of dye "cocktail"
MAP-C	Fixation Addition of nuclear dye Permeabilization Primary antibody addition Secondary antibody addition Cell washes	Fixation Addition of nuclear dye Cell washes
ArrayScan HCS System	Read plates	Read plates

Figure 5: Automating HCS Assays Using a Biomek 2000

In collaboration with our corporate partner, Beckman Coulter, Inc. a study was initiated to automate a series of HCS assay protocols using a stand alone Biomek 2000 and a standard set of pipetting tools, the eight channel wash tool, and supporting peristaltic and vacuum pumps. The goal was to generate validation type plates and therefore Biomek routines were created to accomplish this for three assay types: Cell Motility, Transcription Factor/Kinase Activation, and Mitotic Index. All liquid handling and wash steps were completed on the robot with manual transfer to a CO₂ incubator when required. Valid data, consistent with manual plate preparation, was generated with the Biomek 2000 and an example using Celomics® ERK Activation HiKit Product is shown below.



A scatterplot from a representative "screening" concentration (16.66 micromolar) of compounds tested for p38 MAPK Activation in Swiss3T3 cells. The assay measures accumulation of p38 MAPK cell stress signal in the nucleus of individual cells as it is phosphorylated and translocates from cytoplasmic pools. Clearly, a significant number of these historically toxic compounds activate p38 MAPK. A dose response curve of one of the "hits" from the screen (highlighted in red above) showing a dose dependant activation of p38 MAPK. The inlays show images of representative fields captured during the assay: nuclei are pseudo-colored red and the labeled activated p38 MAPK is colored green, causing the nuclei to appear yellow after translocation.

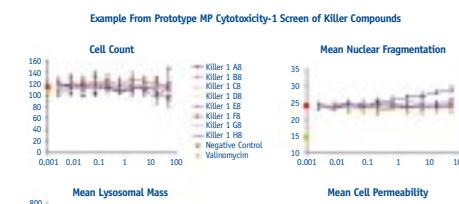


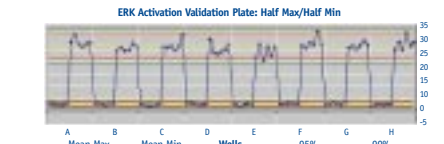
Figure 5 (continued): Representation of the four output parameters simultaneously recorded in the prototype Multiparameter Cytotoxicity-1 Assay using a transformed liver cell line incubated with "killer compounds" for 24 hours. It is important to note that ALL parameters are collected on EACH individual cell imaged. The assay measures: cell count, nuclear morphology changes, lysosomal physiology, and cell membrane permeability using an optimized cocktail of specific dyes. An example plate, in triplicate, from the screen is shown covering 10 point dose response curves for one column of compounds from "Killer Plate 1." In each graph the X axis is the log of micromolar concentrations of test compounds and the Y axis is the measure in the title of each graph. Values for the toxic control compound, valinomycin, are shown as green dots and "no compound" negative control values are red dots on each feature graph. Clearly, several compounds affected lysosomes and nuclear fragmentation in a dose dependant manner.



Top: The Biomek 2000 liquid handling robot and an example screenshot of the "deck layout" for a protocol. Bottom: The ArrayScan HCS System and a screenshot of the Celomics DataViewer visualization software for the ArrayScan HCS System.



Using an ERK Activation HiKit Product and the Biomek 2000, validation type data can be generated. ERK is a signal transduction kinase that translocates to the nucleus when activated with the control compound, anisomycin. Data from both a half maximum/half minimum plate and an anisomycin dose response curve are shown. The max/min plate produced a Z value of 0.41 and the calculated EC₅₀ value for anisomycin was 9ng/ml.



Using an ERK Activation HiKit Product and the Biomek 2000, validation type data can be generated. ERK is a signal transduction kinase that translocates to the nucleus when activated with the control compound, anisomycin. Data from both a half maximum/half minimum plate and an anisomycin dose response curve are shown. The max/min plate produced a Z value of 0.41 and the calculated EC₅₀ value for anisomycin was 9ng/ml.

Conclusions

- HCS HiKit products and protocols are automation compatible.
- Automated HCS assays reduce the variability inherent with manual processing.
- Automated HCS assays produce valid, consistent data.
- Compounds can be effectively screened using automated HCS assays.
- HCS can simultaneously record multiple dose dependent activities a compound may have and cross correlate them down to the individual cell level.
- HCS can be adapted to various platforms including a stand alone Biomek 2000.