

Abstracts

Using the ELx405 Select CW to Wash Loosely Adherent Tissue Culture Cells

Paul Held, Bio-Tek Instruments

The use of cell cultures in large numbers of samples to test agents has now become commonplace in today's HTS environment. Screening assays that utilize cells grown in culture often require one or more wash steps to remove unwanted material or solutions. For example, prior to microscopic examination of fluorescently stained cells, the media needs to be replaced with an inert buffer such as PBS. After fixation, excess formaldehyde is removed prior to antibody staining, after which unbound labeled antibody is removed by washing. These steps require the addition and removal of buffer solution without disruption of the monolayer of cells present in the wells of the microplate. While the majority of cultured cells do not present a problem when they are washed, loosely adherent cells often present problems. Loosely adherent cells do not attach to the microplate substrate very strongly and in many instances they will form loose aggregates or piles rather than spreading in a uniform monolayer. As a result, they are often dislodged when the fluid is dispensed at too high of a pressure by the plate washer, resulting in their loss with subsequent aspiration of the fluid present in the well. To address these problems with washing loosely adherent cells Bio-Tek Instruments has developed the ELx405 Select CW microplate washer. Here we describe the use of the ELx405 Select CW microplate washer to wash the loosely adherent HEK293T cells with PBS in both 96- and 384-well microplates and compare its performance to a standard microplate washer.

Smart lead characterization: Binding Kinetics with Biacore.

Andrew Chow, PhD Applications Scientist at Biacore, Inc.

Abstract: Biacore's label-free detection of molecular binding allows researchers to "visualize" interactions as they happen in real-time. Information from these direct binding studies can provide powerful insights into binding specificity, strength (affinity), and rate (kinetics). Small molecules can be seen binding to protein targets, membranes, and serum proteins providing pharmaceutical scientists with binding profiles, molecular mechanisms, and a comprehensive means of lead characterization. Drug Discovery success stories with Biacore technology will be reviewed.

**The CellLux by Perkin Elmer
Greg Warner**

The CellLux™ is a versatile cellular imaging platform utilizing patented fiber-optic epi-fluorescence imaging technology with broad excitation and emission wavelength ranges enabling a variety of important applications such as Calcium, Membrane Potential and Ion channels. The dual excitation and simultaneous dual emission kinetic FRET capability, instrument sensitivity and the broad excitation and emission wavelength range now enables dyes in the visible and UV spectra to be measured. Ratiometric data will be presented and compared to single excitation and emission dyes demonstrating an increase in the data quality obtained. This flexibility represents a substantial leap forward for conventional calcium and ion channel screening. In addition, we will demonstrate the sensitivity of the instrument allowing for the use of lower concentrations of loading dyes while maintaining assay performance. This in combination with the stand-alone workstation capabilities will help in productivity and cost savings for the end-user. This seminar will discuss the key applications, features, and capabilities of this cell based Epi-Fluorescent Imaging technology.

Enabling Optimized Implementation of High Throughput Protein Expression and Analysis Strategies

Jeremy Lambert, Phynexus.

The majority of today's protein therapeutic companies are driven by a need to effectively identify and characterize large collections of antibodies and proteins with the goal of decreasing time to market of protein therapeutics. Technologies that provide detailed information on protein structure, function, and activity have increased in throughput over the past several years and the trend is expected to continue, however until very recently solutions for high-throughput purification and enrichment of proteins have lagged behind. In this talk we present an integrated approach that includes the automated purification of micro volume protein samples in a 96-well format providing purity and concentration of target proteins for enabling downstream functional characterization using cell-based and biophysical assays.

Cerinox's Tipcharger Technology **Paul Hensley**

Accelerating Chemistry Workflows by automated High Output Technologies

Josef G. Schroer Chemspeed

The use of automated High Output Technologies has expanded from its origins in Biotech and CombiChem into laboratories of many other areas in chemistry and related science. Highly innovative new fields like Catalyst-, Polymer- and Material Science successfully have implemented High Output Technologies to significantly increase their workflow efficiency. The general requirement for increased output and efficiency has boosted the continued evolution of automated laboratory equipment. Herein, we will describe an innovative robotic platform that will not only allow fully automated dispensing of solid and liquids, but also enables the user complete flexibility to configure the platform with the appropriate hardware for the application of his choice.

Automation of Filtration Assays for Drug Discovery Using Positive Pressure

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Filter based assays traditionally use vacuum filtration as a means of separation. The introduction of the Sciclone™ Automated Liquid Handler's Positive Pressure Filtration System allows these types of assays to be run via positive pressure. Examples from ADME and drug screening assays utilizing this technology will be discussed.

For ADME screening the example of aqueous compound solubility using a 96 well MultiScreen® Solubility plate is described. Results show correlation with manual testing methods (including vacuum filtration) as well as a decrease in variability attributed to the use of automation.

For drug screening, receptor and protein kinases assays, which address the most important targets for drug discovery, are outlined. To date, most of these assays have been performed on 96-well harvest platforms. The recent availability of 384 well filter plates and automation expands the utility of heterogenous binding assays resulting in higher throughputs and reduced reagent costs. GPCR & kinase applications using 384 well filter plates are discussed to demonstrate that automated quantitative screening assays can be developed whose performance is not only equivalent but superior to 96-well formats as measured by Z' values.

Posters

Title: Synergy HT, A Robotic Compatible Microplate Multidetector Reader Designed for Today's Luminescence Research Needs

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Genetic reporting assays are widely used to study gene expression and cellular responses to external stimuli in prokaryotic and Eukaryotic organisms. Dual-reporter assays, as the name implies entails the use of two independent reporter systems simultaneously to improve experimental accuracy. While many different technologies exist to perform dual reporter assays, luminescent assays have generally been utilized because of their ease of use, sensitivity, and low background levels. To address diverse assay needs, Bio-Tek has introduced the Synergy HT Multidetector Microplate Reader. This robotic compatible multidetector reader, when configured with injectors is capable of performing either glow or flash luminescent assays in microplates. Each injector uses inert tubing and a replaceable stainless steel injector tip. Detection is accomplished with a sensitive PMT. In this study, the Synergy HT was used to perform both Dual-Glo and flash Dual-luciferase® assays™. Detection limits for Firefly and Renilla luciferase, as well as several performance parameters are reported.

Dual-Luciferase is a trademark of Promega Corporation and is registered with the U.S. Patent and Trademark Office. Dual-Glo is a trademark of Promega Corporation.

Convenient , accurate and precise dispensing of 95% of catalogue solid chemicals.

Author: Thomas Stiglic, Chemspeed.

The Solid Dispensing Unit (SDU) on the Chemspeed's Accelerator VLT 100 has the capabilities to dispense solids as conveniently and accurately as liquids. It features a built-in balance that allows for dispensing into virtually any vial on the platform independent from its location.

High-Throughput Automation of a Dual Reporter Assay in Low-Volume 384 & 1536-Well Plate Formats using the Deerac Fluidics' Equator™ NS 808 - Eight Tip Pipetting System, Promega's Chroma-Luc™ Technology, and BMG LABTECH's PHERAstar Microplate Reader.

David Lorenz, Aoife Gallagher, Brad Larson (Promega Corporation), Tracy Worzella (Promega Corporation), Michael Bjerke (Promega Corporation), and Eric Matthews (BMG Labtech)
Deerac Fluidics

The drive towards miniaturization within the pharmaceutical and biotechnology fields has created a need for liquid handling technologies that accurately deliver low volume reagents to high-density plates. This has also created a need for simple, fully scaleable assays in low volumes. Here we demonstrate the successful combination of both through the use of the Equator™ NS 808 - Eight Tip Pipetting System, and the dual color Chroma-Luc™ technology. Cellular lysates, containing the green *CBG99luc* and red *CBRluc* genes, followed by Chroma-Glo™ reagent, were dispensed in low-volume 384, and 1536-well formats using volumes ranging from 10ul to 500nl. Luminescence from the two luciferases was then simultaneously measured using the BMG LABTECH PHERAstar plate reader. The exceptional Z' Factor scores, linearity, limit of detection, and separation of signal data, show the flexibility and reliability of the Equator™ NS 808, and Chroma-Luc™ dual reporter technology in any high-throughput situation.

Rapid Analysis of 7-Hydroxycoumarin Glucuronide Using Micro Parallel Liquid Chromatography

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The development of *in vitro* assays to estimate potential drug-drug interactions related to UDP-glucuronosyltransferases (UGTs) is a topic of great current interest.¹⁻⁶ The development of a simple, rapid and convenient assay for the study of these interactions is highly desirable.⁷ Recent work has reported the use of 4-trifluoromethylumbelliferyl (TFMU) as a marker substrate, but the speed of this assay is compromised by the use of standard HPLC.⁸ In this work we use an analogue of TFMU, 7-hydroxycoumarin (7-HC), to demonstrate the rapid analysis of 7-hydroxycoumarin glucuronide (7-HCG) in the presence of 7-HC using micro parallel liquid chromatography. The method is short (7 minute gradient, total run time of 14 minutes), and the use of fluorescence detection avoids interferences (Figure 1). The calibration curve for quantifying the concentration of 7-HCG in the assay is linear, reproducible (Figure 2) and easily passes quality control acceptance criteria. Micro parallel liquid chromatography increases sample throughput 24-fold, and subsequent collection of isolated fractions permits an interface to mass spectrometry for confirmation of signals. These results demonstrate how micro parallel liquid chromatography can provide a path to a simple, convenient and *rapid* assay for the study of drug-drug interactions related to UGTs.

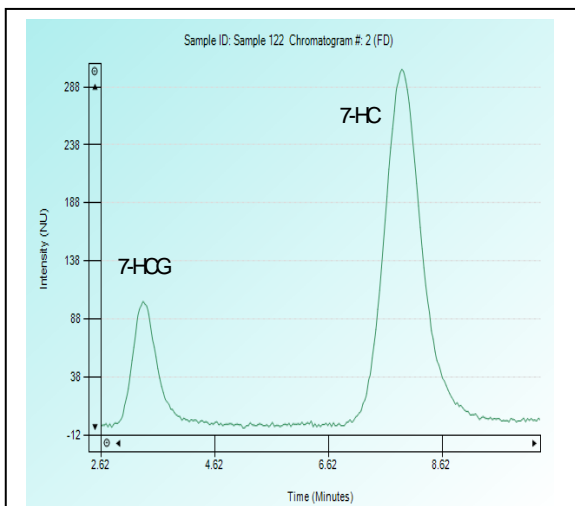


Figure 1. Χηρωματογράμ οφ 0.5 μM 7-HCG with 0.0385 μM 7-

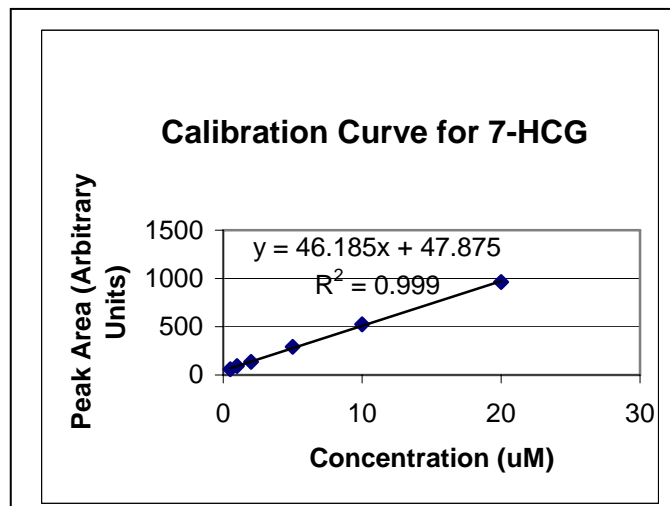


Figure 2. Standard curve for 7-HCG (concentration range 0.5 μM to 20

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High-Throughput LC-based Assays for Screening of Caspase Inhibitors

Jeff Koehler, Courtney Coyne

Nanostream

Separation-based assays performed using liquid chromatography offer several advantages when compared to assays performed using plate readers. LC-based assays reduce interference by separating quenchers and fluorescent compounds from the substrate. Also, simultaneous detection by UV absorbance and fluorescence provides additional assay information that is not available from a plate reader. While the throughput of conventional LC instrumentation limits its value for assay detection, micro parallel liquid chromatography (μPLC) increases analytical throughput by permitting analysis of 24 samples at the same time.

The Nanostream® Veloce™ μPLC system—used in conjunction with 24-column Brio™ cartridges—facilitates assay development, offers quantitative information about hits, and provides additional information on compound purity and solubility. The Veloce system enables scientists to incorporate separations prior to

detection when performing screening studies. This poster demonstrates the utility of the Veloce system for high-throughput LC-based assays for screening of caspase inhibitors and compares results to those obtained using a plate reader.

A comparison of sample preparation methods as measured by LDH activity, nucleic acid yields, sample homogeneity, and sample throughput.

Andoria Tjondrokoesoemo¹, Andrew I. Brooks², Charles Grant³, and David Burden¹

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The release of analytes from biological tissue is one of the first processing steps post-harvest. To do this, a myriad of tools are available for disrupting samples, however it is not always clear as to the strengths and limitations of these tools. In high throughput environments, where each sample cannot be readily scrutinized, it is fundamentally important that the disruption step is uniform and does not drastically increase the variability of sample analysis. In order to assess the effectiveness of sample disruption, our team examined the tools and handling options on a variety of biological samples, including cultured cells, bacteria, yeast, liver, muscle, heart, lung, and plant tissue (seeds and leaf punches). Disruption methods employed included liquid shearing, sonication, cryogrinding, bead beating and mechanical homogenization. Disrupted samples were measured for lactate dehydrogenase release as determined with an INT assay, yield of nucleic acids, resulting particle size and homogenate consistency, and ease of handling of homogenate with a Biomek FX Assay Workstation. Generally the efficiency of sample disruption was inversely proportional to its throughput, the exception being with high throughput bead beating. Manual glass homogenizers can process large samples to generate suspensions that have good LDH activity, extremely small particle sizes, and are readily liquid handled. However, throughput was laboriously slow and residual fibrous tissues often remained adhered to the homogenizer. Mechanical homogenization yielded very fluid samples, even of tough tissues such as muscle however resulting particle sizes (observed microscopically) were relatively large. Sample debris cleared by centrifugation retained considerable enzyme activity. Sonication was effective for disrupting small particles, but yield poor results on solid samples. Sonication, though relatively fast, requires a prior disruption step for any solid samples. High throughput bead beating (mixer mill) was very effective at disrupting samples, yielding the highest LDH activity. However, sample size was limited to 100 mg/well using deep well plates. Cryogenic grinding was also examined as a method for preparing samples for repeated handling. Frozen tissue powder could be measured and subsequently homogenized to yield high levels of LDH activity and nucleic acids. A matrix of biological tissue versus sample disruption method is discussed. **Conclusion:** All methods used to disrupt samples have strengths and weaknesses. Lower throughput methods can be used to process

larger samples with good results. High throughput methods are also effective, but sample size and type may be a limitation. Cryogrinding of tissue simplifies repeated handling of samples and aids in overall processing.

Monoclonal Antibodies

BioVeris

The use of antibodies for diagnostic, clinical and epidemiological assays has highlighted the utility of immunoassays for agents of bioterrorism and infectious diseases. The development of monoclonal antibody producing lines has traditionally been a scientist labor-intensive process, incorporating numerous cell culture, screening and selection steps. As antibodies for a newly defined target are needed, scientists may spend a great deal of time generating a cell line that produces a reliable source of the new antibody.

By utilizing liquid handling automation coupled with automated cell culture incubators and custom software, a large portion of the repetitive tasks associated with monoclonal antibody development have been alleviated. The use of robotic handlers has enabled incorporation of electrochemiluminescence based screening methods into the process. This was accomplished within a sterile environment to permit the cell culture processes to be intermingled with non-sterile aspects such as screening, moving the process much closer to a fully automated platform. The processes by which cell lines are manipulated, tested and maintained, active producers are screened and the clones of interest are selected are discussed.

Improving Automated Liquid Handler Performance through Reliable Volume Delivery Measurements

Artel

Automated liquid handling (ALH) systems are highly effective at increasing throughput and decreasing labor expenditure in a number of applications such as drug discovery and development, proteomics, genomics, and molecular diagnostics. Significant technological advancements over the past decade have led to broad implementation of ALH equipment. However, despite the technological sophistication of modern ALH systems, they are still fallible devices requiring regular performance verification. Additionally, optimum performance of volume delivery is attainable for ALH devices only when a reliable measure of the delivered volume can be made, from which appropriate protocol adjustments can be carried out to tune the device performance.

The dual-dye photometric approach implemented by the Artel Multichannel Verification System (MVSTM) provides a tool for measuring both the accuracy and precision of volume delivery from various types of multichannel liquid handling equipment (both automated instrumentation as well as manual pipettes). The traceable measurements provided by the MVS system can be used to reliably analyze ALH performance, as well as adjust delivery protocols to

optimize the system. This presentation will focus on case studies wherein ALH performance was measured using the MVS, and optimized for ideal volume delivery. Additionally, recent developmental work for extending the MVS testable volume range down to 0.1 μL , along with system validation in this enhanced volume range will be discussed.