

Bench top solutions for cell based assays.

WHITE PAPER



BENCH TOP SOLUTIONS FOR CELL-BASED ASSAYS



SPARK®
Multimode Reader



HYDROSPEED™
Microplate Washer



D300E
Digital Dispenser

COMMON PROBLEMS WITH CELL-BASED ASSAYS

Cell-based assays are very popular in life sciences research, as they represent an *in vitro* system which offers results comparable to *in vivo* systems. However, this improved biological relevance is counterbalanced by the complexity of working with a living organism. Cell-based assays therefore require special precautions and care when planning and conducting experiments, including:

- **Stable environmental conditions**
 - Temperature
 - Gas (CO₂/O₂)
 - Humidity
- **Avoiding contamination**
 - Bacteria
 - Fungi
 - Mycoplasma
- **Constant monitoring of the cells**
 - Health
 - Number
 - Shape
- **Protection against harmful influences**
 - Metabolic waste products
 - High DMSO concentrations

In most cases, experimental set-up is a compromise between scientifically optimal and realistically achievable workflows. Results of cell-based studies are therefore subject to environmental bias from the experimental design. Prominent examples include overnight measurement gaps in long-term kinetics and environmental stress from cycling of the cells between the reader and incubator.

Tecan understands these issues, and has optimized its product portfolio to meet the requirements of working with cells. Our products offer many features designed to help scientists avoid error-prone workflows, minimize artefacts and generate more relevant and reproducible data in shorter time. In this white paper, we will demonstrate how our benchtop instruments – including the Spark multimode reader, HydroSpeed microplate washer and D300e Digital Dispenser – can help you to produce better results, faster and more easily.

SIMPLIFIED CELL-BASED WORKFLOW



Cell counting



Cell seeding



Cell growth



Medium Exchange

Cell counting
module

Injector module

Temperature control
Humidity control
Gas control
Confluence
determination

Cell protection
Tunable aspiration
Drop-wise dispensing



Compound addition



Assay incubation

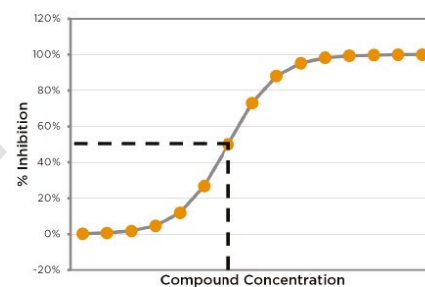


Assay measurement

Rain of pl droplets
Minimal DMSO
addition
Randomization
Fast process times

Temperature control
Humidity control
Gas control
QC picture

Z-optimization
Optimal Read
function
Bottom reading
function
Te-Cool

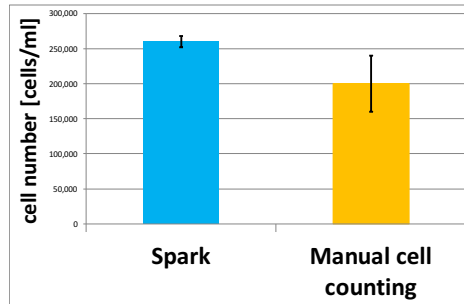


CELL COUNTING AND NORMALIZATION

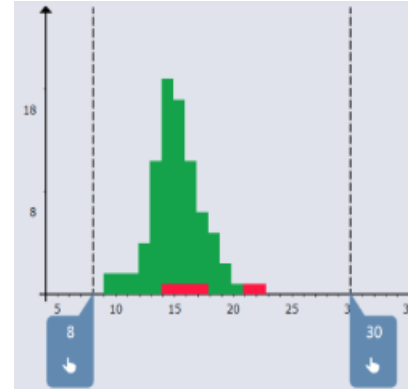
The first step of a cell-based assay is usually counting the number of cells present to calculate the dilution factor required to produce a normalized seeding suspension. The Spark's cell counting module uses bright field imaging to offer a fast and reliable counting of up to eight individual samples using Tecan's disposable Cell Chips™ and Cell Chip Adapter.



The Cell Chip adapter can measure eight separate samples



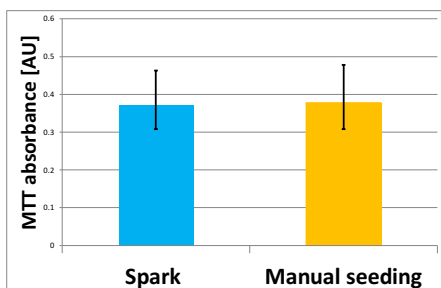
Comparison of automated and manual cell counting



Histogram of a trypan blue-based live/dead count (green = alive, red = dead)

CELL SEEDING

Spark's injector module allows cells to be automatically seeded into microplates with up to 384 wells. Its integrated stirring and heating functionalities keep cell suspensions homogenous and at a stable temperature, allowing reproducible seeding of healthy cells at both high densities - for immediate assays or treatments - or at lower densities for further growth or microplate-based growth studies.

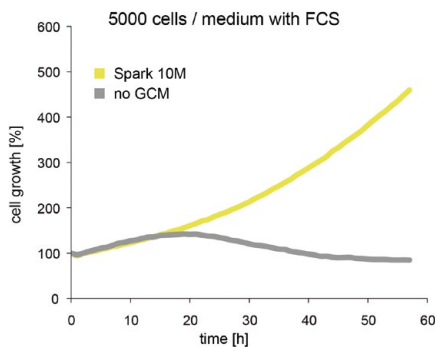


Comparison of automated (blue) and manual (yellow) cell seeding, using an MTT assay to determine cell viability

CELL GROWTH

Spark offers all the features necessary for optimum cell growth of eukaryotic cells or bacteria within the reader. Temperature, gas partial pressures (O₂ and CO₂), humidity and shaking can be controlled to enable long-term growth studies, kinetic measurements or workflow automation over several days without manual intervention.

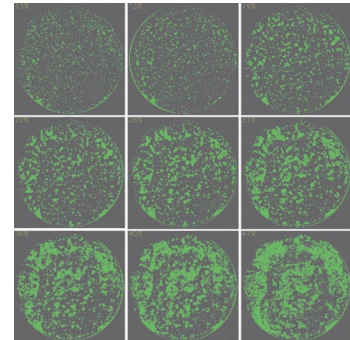
Spark's imaging module enables automated acquisition of bright field images for each well. This allows the system's SparkControl™ software to calculate the confluence status for each well, as well as automatically perform specific activities – such as addition of a compound via the injector module – once a user-defined confluence threshold is reached. Images can also be easily viewed for quality control and troubleshooting in case of unexpected assay results.



Growth curve for eukaryotic cells with (green) and without (black) gas control



Innovative Humidity Cassette reduces buffer or medium evaporation



Confluence determination for A431 cells over 48 hours, with color overlay representing the area recognized as cells

MEDIUM EXCHANGE

The HydroSpeed microplate washer enables gentle and reproducible medium exchange, using tunable aspiration settings and Cell Protection™ drop-wise dispensing to minimize cell detachment. To ensure reproducible performance, the HydroSpeed's Anti-Clogging™ function automatically performs rinsing and soaking steps when the washer is idle. The wash head can also be easily removed for cleaning using an ultrasonic bath, etc.



Wash procedure	HydroSpeed plate washer with 96i head	Manual washing
A431 cells		
Cell viability [%]	80.3	18.7
Wash efficiency [%]	95.6	98.9
P815 cells		
Cell viability [%]	73.0	10.5
Wash efficiency [%]	95.4	97.3

Comparative results for cell viability and wash efficiency of adherent (A431) and very weakly adherent (P815) cells using the HydroSpeed plate washer and manual washing.

COMPOUND ADDITION

Compound addition is usually the most labor-intensive step of any cell-based assay. The D300e Digital Dispenser's easy-to-use software – D300eControl – speeds up this process by automatically calculating the dispense volumes and concentrations required to achieve the desired dose-response curve. High speed, contact-free dispensing of picoliter volumes allows 96 doses to be dispensed in around one minute – directly from a stock solution. This minimizes the amount of time the cells spend outside of the incubator and reduces the risk of temperature shocks. The low dispense volumes of typically few hundred nanoliters minimizes the volume increase and makes volume reduction before compound addition obsolete.

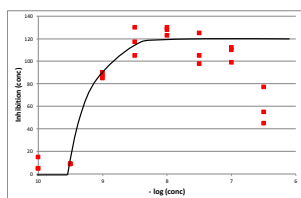


For small molecules in DMSO, the compound stays in 100 % DMSO until it enters the assay plate, eliminating predilution steps and the associated risk of precipitation. Drop-by-drop direct dispensing also minimizes the amount of DMSO added to each well and avoids localized toxic build-up, which usually have negative effects on cells.

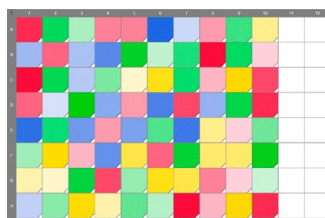
By allowing any dose to be delivered to any well, the D300e allows advanced assay patterns and one-click randomization to minimize edge effects. Using the D300eControl software, assay results can easily be converted back into a non-random layout. Additional functionalities – such as buffer back fill, targeted titration and synergy titration – make the D300e a powerful tool for setting up dose-response curves.



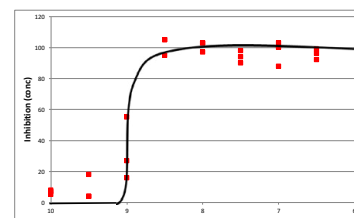
Non-random plate layout



Assay result from non-random plate layout



Randomized plate layout



Assay result from randomized plate layout

ASSAY INCUBATION

Many cell-based assays rely on kinetic measurements or growth studies, with repeated measurements over many hours or days. This requires a significant commitment from researchers in terms of time and scheduling to cycle the plate between reader and incubator. Spark enables the plate to remain in the reader, with 'incubator-like' control of conditions such as temperature, gas and humidity. This allows continued measurements without overnight gaps, and avoids artefacts created by moving the assay plate between reader and incubator. The system's Humidity Cassette – which has water reservoirs to maintain a humid environment – also helps to minimize evaporation of the cell culture medium, with an integrated Lid Lifter™ to remove and replace the cassette lid for measurements or compound additions.

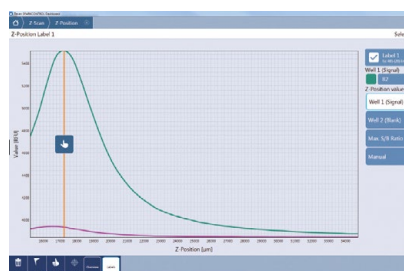


This set-up, combined with Spark's bright field imaging module, can be used to perform growth or migration studies, monitoring the position or confluence status of cells in each well. In addition, SparkControl allows automation of complete assay workflows, such as GFP-tagged protein expression studies:

- Cell incubation within the reader, using a bright field imaging kinetic to monitor cell growth
- Automated reagent addition with the injector module to start protein production once a predefined cell density is reached
- Start of a fluorescence kinetic in addition to the imaging kinetic to monitor protein expression and a cell proliferation

ASSAY MEASUREMENTS

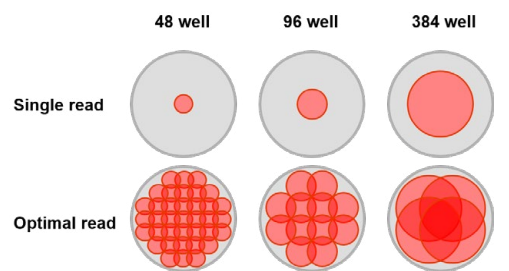
Measurement of assays using adherent cells can be complex. Signals originate only from the narrow layer of cells at the well bottom, autofluorescence of the medium or inhomogeneous distribution of the cells. Spark's automated Z-focusing maximizes signal intensity by adjusting the measurement focus to the location of the cells. This is complemented by the system's bottom reading measurement mode, which minimizes the distance the light has to travel through the medium to reduce autofluorescence. Spark also offers an Optimal Read function, which performs multiple measurements at defined points across each well, providing more representative results for inhomogeneous cell layers.



Z-focusing for adjusting focal point of signal detection and maximizing signal to blank ratio



Bottom read for signal detection from below the well to minimize noise from the cell medium



Optimal read for signal detection at multiple positions in the well to obtain a representative signal

OVERALL BENEFITS

Cell based assays allow researcher to study processes in the context of a living organism. The biggest challenge in such assays is keeping the cells in a healthy environment where only the intended treatment drives the assay result and not uncontrolled environmental influences. Tecan's bench top instrumentation is designed with the unique challenges of cell biology laboratories in mind.

Spark offers cell seeding, optimal growth, incubation conditions and automated growth control combined with the functionalities of a high performance multimode plate reader. HydroSpeed is an easy to handle plate washer meeting highest standards in terms of performance and reliability. The D300e digital dispenser combines pl dispensing capability with high speed and flexibility to eliminate the need for serial dilution and minimize the time spend on dose response curve set up. Overall, the trilogy of Tecan's benchtop solutions for cell biology research – Spark, a high-performance multimode reader designed with the needs of cell biology labs in mind; Hydrospeed – A gentle and easy to use washer platform for your cells; and D300e – A low volume digital dispenser, can empower labs to overcome environmental artefacts and generate results with confidence.

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