Validation of the Byonoy Reader with the InstaCELL Cytotoxicity Kit

A new generation of microplate absorbance readers captivates with their small footprint and innovative set-up. Within seconds, a compact system without moving parts composed out of 4 LEDs and 96 photodiodes allows the simultaneous reading of the individual signals from a 96-well plate. The built-in assay protocol is performed by means of a software which controls the reader via USB. Here we demonstrate the adequacy of the reader for typical cell-based applications like cytotoxicity testing or proliferation assays. The ultra-compact absorbance reader is particularly useful for standardized routine assays, as it provides high robustness and reliability, while minimizing susceptibility to errors.

introduction

Cellular models are an indispensable and well-established tool in basic research, target verification and drug discovery. Over the last few years, cell-based assays became of high importance also in more regulated fields of pharmaceutical development such as safety assessment, where cellular approaches can substitute animal testing, or the quality control of therapeutic proteins, for which bioassays are the method of choice to evaluate the potency of a marketed drug.

For GLP/GMP testing, the requirement for accuracy and reproducibility of cell-based assays is significantly higher than for research applications. To meet this on the cell level, assay-ready cells, which are used instantly after thawing like a reagent without prior cultivation, proved to increase assay precision. However, when it comes to the measurement of the results, the adequacy of a multimode reader for an assay can become extremely challenging.

Fig. 1: Absorbance 96. Byonoy's innovative, ultracompact microplate reader.

To overcome this limitation, Byonoy has developed a new generation of microplate readers with an innovative set-up. Within seconds, 96 detection units simultaneously measure the individual signals of the samples in a 96-well format (Fig. 1). Furthermore, no moving parts are involved and an automatic calibration is included as part of the customer-oriented design. The built-in assay protocol is executed by a software which automatically controls the reader. Assay failure by incorrect user manipulation is mostly excluded.

Demonstrated here for a frequently used XTT cell viability assay, the combination of assay-ready cells, instaCELL® assay kits and preset plate readers can provide the high reliability and precision required for applications in a regulated environment.

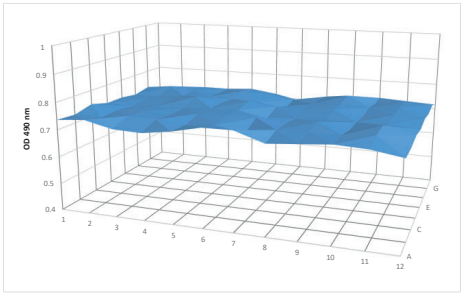

Fig. 2: Reader accuracy. Surface plot of a well to well comparison with XTT measurement.

reader accuracy

30.000 L-929 cells per well were seeded in a 96-well plate and incubated for 24h at 37°C in a humidified atmosphere. After this, XTT was added and incubated with the cells for 4h at 37°C. Since XTT is metabolized by living cells to a deep orange formazan salt, the measured OD is proportional to the cell viability. In order to test the accuracy of the instrument and the individual 96 photodiodes, the supernatants were pooled and dispensed into a fresh 96-well plate, which was next inserted into the Byonoy plate reader and measured at 492nm (Fig. 2). Results showed highly even absorbance levels with an extremely low SD of 1.2%. In addition, no outliers were detected, indicating an accurate measurement of the single photodiodes.

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assay kinetics

L-929 cells were seeded into a 96-well plate at different densities ranging from 1.000 to 100.000 cells/well. After an incubation of 24h at 37°C to guarantee cell adhesion, XTT was added to quantify the metabolic activity of the cells. With the Byonoy reader the absorbance kinetics were acquired over the course of four hours to determine the best incubation time and cell seeding density for the assay (Fig 3).

assay accuracy

A reproducible and routine use of cells requires proper and well-standardized cultivation procedures. To minimize cell culture related variances, assay-ready cells were proven to be a valuable tool. These cells are cryopreserved at a highly viable and functional state and can be used in assays like a typical reagent without any prior cultivation. Based on this expertise, the instaCELL Cytotoxicity Assay Kit has been designed to improve the reliability and reproducibility in routine toxicity testing. It contains assay ready L-929 cells, recovery buffer and assay medium, an assay plate, the XTT reagent and suitable positive and negative controls.

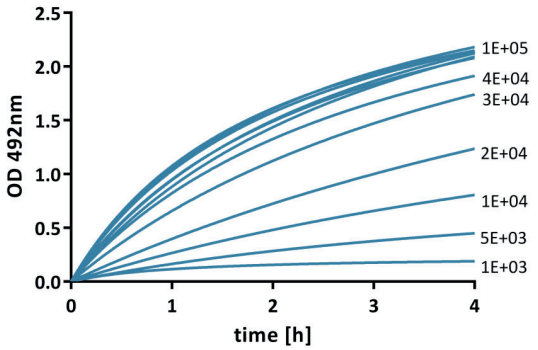
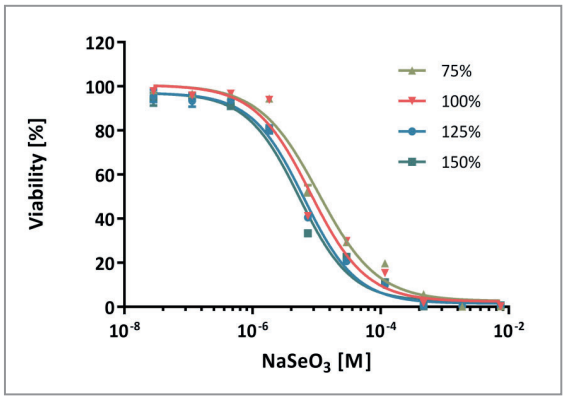


Fig. 3: Assay kinetics. Metabolization of XTT over time in relation to the cell density.

To test cytotoxicity according to the instaCELL kit, a vial of assay-ready L-929 cells was quickly thawed in a water bath at 37°C and the cell suspension transferred into 8ml prewarmed RPMI. The cells were pelleted at 80xg for 3min, resuspended in 10ml assay medium (RPMI 1640, 10% FBS, 2mM L-Gln) and seeded at 30.000 cells/well in a 96-well plate. Next, four sets of serial dilutions from a toxic reference substance (sodium selenite) were prepared as 75%, 100%, 125% and 150% of the nominal concentration, added to the cells and incubated at 37°C in a cell culture incubator. After 24h XTT was added to the cells for 4h and the metabolic activity (viability) of the cells was determined by measuring the absorption at 492nm. Finally, the IC_{50} value of the four dilutions was determined. Results displayed an almost perfect match to the calculated value of the dilutions, thereby demonstrating the accuracy of the assay (Fig. 4).



	75%	100%	125%	150%
IC_{50}	1.07E-05	8.06E-06	6.37E-06	5.36E-06
Slope	-0,945	-1,000	-1.058	-1,064
Top	100.9	100.5	96.57	95.15
Bottom	2,591	2,381	1,874	2,894
IC_{50} in %	**75,3**	**100**	**126,5**	**150,4**

Fig. 4: Assay accuracy. 75%, 100%, 125% and 150% of the nominal concentration of serial dilutions were tested to determine the accuracy of the measured IC_{50} values.

discussion

On the one hand, the new microplate absorbance-based reader by Byonoy appeared to be a convenient and robust instrument. Due to the simultaneous measurement of 96-wells, the read-out is very quick and even allows the acquisition of fast kinetics. Furthermore, the 96 photodiodes were able to accurately and sensitively detect 1.000 cells in the XTT assay. On the other hand, the use of assay-ready cells in combination with the Byonoy reader resulted in an exceptionally high accuracy due to the exclusion of typical factors inducing assay variability in this set-up, e.g. cell culture handling and microplate reader settings. Particularly, for routine GMP/GLP assays, where assay precision and accuracy are critical, the use of Byonoy's robust reader, preset for a defined application, in combination with prequalified assay-ready cells, can improve the reliability and reproducibility of any given cell-based assay.

related products

DE048 Byonoy plate reader (absorbance)
SF021 instaCELL® cytotoxicity assay kit (XTT)