acCELLerate

Validation of the Byonoy Reader with the InstaCELL Cytotoxicity Kit

A new generation of microplate absorbance readers captivate by their small footprint and an innovative set-up. Within seconds, 96 LEDs and 96 minicameras simultaneously read the individual signals of a 96-well plate. There are no moving parts involved. The built-in assay protocol is executed by a software which controls the reader via USB. Here we demonstrate the qualification of the reader for typical cell-based applications like cytotoxicity testing or proliferation assays. The ultra-compact absorbance reader is in particular useful for standardized routine assays, as it provides a very high robustness, reliability and a very low susceptibility to errors.

Fig. 1: compact, small, no switches.
The microplate reader from Byonoy.

introduction

Cellular models are an indispensable and well-established tool in basic research, target verification and drug discovery. Since a couple of years also in the more regulated fields of pharmaceutical development cell-based assays became of high importance. In toxicology, test on cells can substitute animal experiments. In quality control of therapeutic proteins, bioassays are the method of choice to test the potency of the marketed drug.

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

ByoNoy has developed a new generation of microplate readers with an innovative set-up. Within seconds, 96 LEDs and 96 minicameras simultaneously read the individual signals of a 96-well plate (Fig. 1). There are no moving parts involved and no calibration required. 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 which is required for applications in a regulated environment.

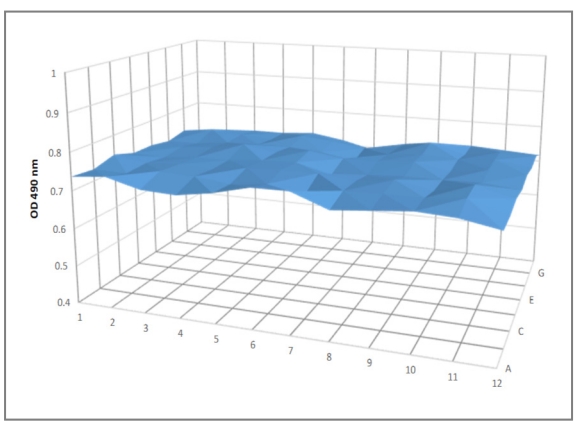

Fig. 2: Reader Accuracy. Surface plot of a well to well comparison with XTT measurement.

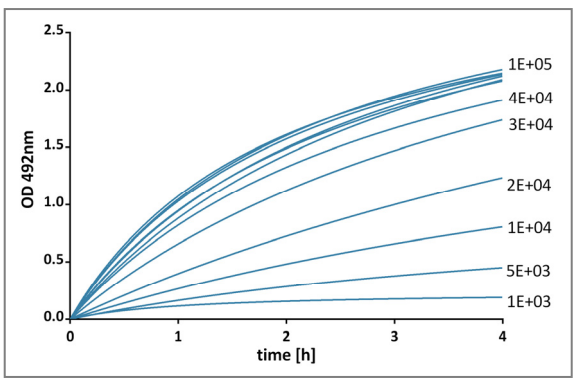

Fig. 3: Assay Kinetics. Metabolization of XTT over the time in relation to cell density.

reader accuracy

30.000 L-929 cells were seeded into the well of a 96-well plate. The cells were incubated for 24h at 37°C in a humidified atmosphere before XTT was added. XTT which becomes metabolized by living

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cells to a deep orange formazan salt. The OD is proportional to the viability of the cells. In order to test the accuracy of the instrument and the individual 96 miniature cameras the supernatants were pooled and dispensed into a fresh 96-well plate. The plate was inserted into the Byonoy plate reader and measured at 492nm (Fig. 2). The absorbance was very even with a very low SD of 1.2%. No outliner were detected indicating the accurate measurement of the 96 mini cameras.

assay kinetics

L-929 cells were seeded into a 96-well plate at different densities between 1.000 and 100.000 cells per well. The cells were incubated for 24h at 37°C to adhere and XTT was added to quantify the metabolic activity of the cells. The plate was inserted into the Byonoy reader and absorbance kinetics were acquired over a course of four hours to determine best incubation time and cell seeding density for the assay (Fig 3).

assay accuracy

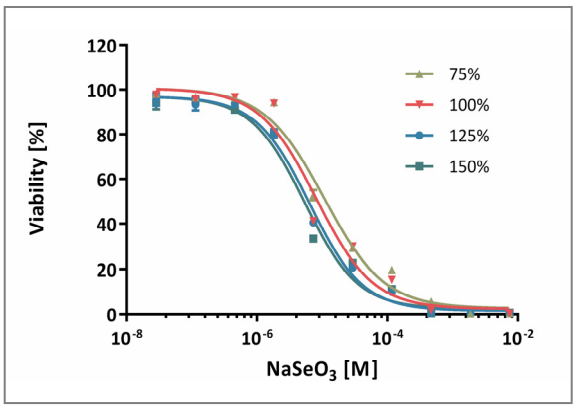
A reproducible and routine use of cells requires proper and well standardized cell culture procedures. To minimize cell culture related variances, assay ready cells proved to be a valuable tool. Assay ready cells are cryopreserved at a highly viable and functional state and can be used in assays like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

A vial of assay ready L-929 cells was quickly thawed in a water bath at 37°C and the cell suspension was transferred into 8 ml prewarmed RPMI. The cells were pelleted at 80xg for 3 minutes and resuspended in 10ml assay medium (RPMI 1640, 10% FBS, 2mM L-Gln). 30.000 cells were seeded int each well of a 96-well plate.

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 24 hours XTT was added to the cells for 4 hours and the metabolic activity (viability) of the cells was determined by measuring the absorption at 492nM. The IC_{50} value of the four sets of dilutions were determined which almost perfectly matched the calculated value of the dilutions, demonstrating the accuracy of the assay (Fig. 4).

discussion

The new microplate absorbance reader from Byonoy appeared to by easy to use and robust instrument. Because of the simultaneous measurement of 96-wells the read-out was very quick and allows the acquisition even of fast kinetics. Nevertheless, the 96 mini cameras work very accurate and sensitive able to detect 1.000 cell in XTT assay.



	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% 150% of the nominal concentration of serial dilutions were tested to determine te accuracy of the measured IC50 values.

The use of assay ready cells which are applied in cell based assays without prior cultivation in combination with the Byonoy microplate reader result in a very high assay accuracy, because typical reasons for assay variability, e.g. cell culture handling and microplate reader settings are systematical excluded for the assay in this set-up. In particular for routine GMP/GLP assays where assay precision and accuracy are critical determinants, the use of Byonoy's robust plate 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)