

Novel Homogeneous Assay System for Predictive Toxicology Screening

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ABSTRACT

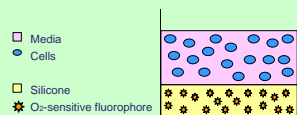
We have developed a novel, homogeneous, fluorescent assay system ideally suited for the throughput-oriented screening environment commonplace in most modern pharmaceutical development groups. The BD™ Oxygen Biosensor System (BD™ OBS) is based upon an oxygen-sensitive fluorescence technology for detecting cell viability. Because this system eliminates the need for subsequent reagent addition steps, potentially hazardous reagents, and time-consuming incubation periods, it addresses several of the throughput limitations inherent in more traditional methods, such as MTT, and ³H-thymidine uptake. Tests of this assay system with a variety of common drugs and both microbial and mammalian cell lines reveal that it is broadly applicable. A series of kinetic assays comparing the cytotoxicity of several commercially available compounds was run side-by-side with both the BD ViaSante™ Oxygen Biosensor System and with standard MTT. Both systems gave comparable IC₅₀ values for each drug. Since our system requires neither subsequent reagent addition steps nor incubation periods, it allows for repeat measurements over time on the same wells, thereby greatly simplifying and enhancing the collection of kinetic cellular toxicity data. The kinetic plots resulting from experiments utilizing HL60 cells and 5 common drugs are consistent with the known modes of action of the drugs, demonstrating the potential of our system for conducting predictive “toxicokinetic” screens.

INTRODUCTION

Assessment of a drug candidate for cellular toxicity is a key aspect in the drug development process. Current methods (e.g. MTT, ³H-thymidine) for screening cellular toxicity, while widely used, were not designed for use in a throughput-oriented screening environment. As such, they possess many undesirable properties (multiple steps, the need for caustic solvents, incubation periods, hazardous/radioactive waste disposal, etc.). Moreover, because such “end-point” assay protocols are not amenable to kinetic assays, the information they can provide is limited to an estimation of the toxic concentrations of the drug candidates. Such one-dimensional assays run counter to current trends in secondary screening toward quicker, more reliable, and more information-rich assays for ADME-Tox. The BD OBS addresses the throughput limitations, while simultaneously allowing users easily to upgrade a traditional toxic screen by “front loading” additional mechanistic predictive capability.

The BD OBS system incorporates an oxygen-sensitive ruthenium-based fluorophore into a gas-permeable silicone matrix. Under ambient dissolved oxygen concentrations, the fluorophore’s fluorescence is largely quenched. As cellular respiration depletes the local oxygen concentration in the well, the fluorescence increases.

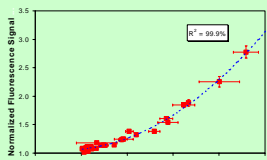
How Does It Work?



The BD Oxygen Biosensor system consists of a fluorophore embedded in a silicone matrix that is permeable to oxygen.

BD™ Oxygen Biosensor System

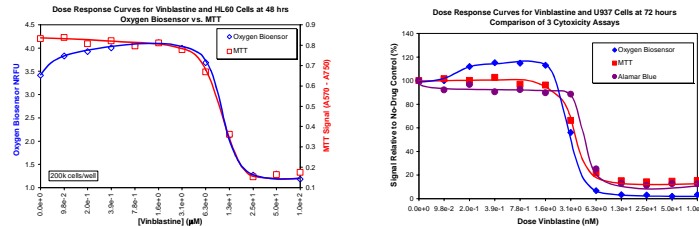
Quantitative Correlation Between Signal and Cell Number



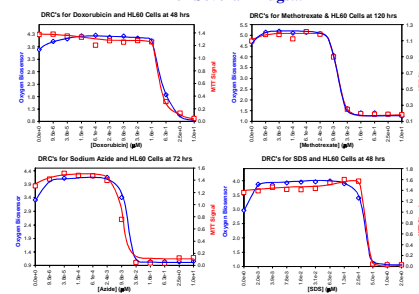
As the concentration of oxygen is depleted in the polymer matrix, through cellular respiration or enzymatic reaction, the fluorescence increases. Signal correlates directly to extent of cellular or enzymatic oxygen consumption.

No reagents needed to generate signal

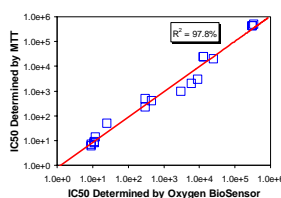
Determines Same IC₅₀'s as Traditional Methods



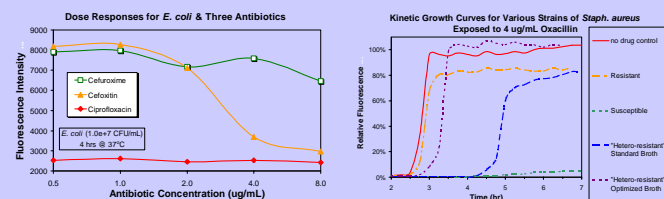
For Several Drugs...



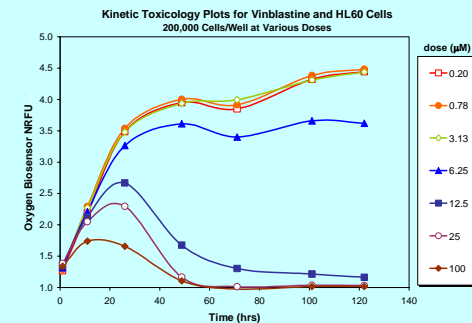
...With Excellent Correlation



It works with bacteria, too...

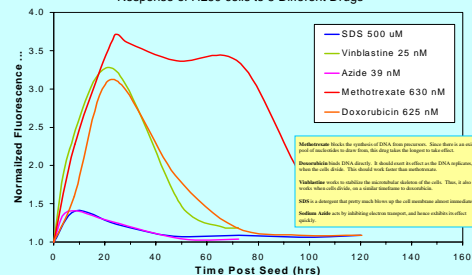


Kinetic “Time-to-Death” Profiles Give Predictive Tox Insight



BD Oxygen Biosensor System Comparative & Predictive Kinetic Tox Profiles

Response of HL60 cells to 5 Different Drugs



CONCLUSIONS

- Respiring cells consume oxygen and give rise to fluorescent signal proportional to cell number, without the need for additional reagents.
- As cells die due to toxic effects, oxygen consumption drops off and the fluorescence decreases.
- IC₅₀ values determined for several drugs and with several cell types correlate closely with values obtained via traditional protocols.
- Ability to read repeatedly enables kinetic analysis.
- Kinetic toxicology profiles reflect known modes of action for the drugs studied, adding a valuable predictive element to toxicology screening.