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## Introduction

Using the BD™ Oxygen Biosensor System (BD OBS) is straightforward and simple to determine the growth kinetics of cells in culture, including a doubling time. Using this single parameter allows for easy comparison of various growth conditions or experiments, and can greatly facilitate applications as diverse as media optimization and quantitation of microbial drug resistance.

The experiment to determine doubling time simply entails allowing a range of seed densities to proliferate. By taking repeat readings of the BD OBS, you will generate a series of growth curves, similar to those shown in *Figure 1*.

Such a set of nested sigmoidal growth curves is characteristic for a proliferation experiment such as this, and has been observed for mammalian cells, bacteria and fungi. The only difference between the various cell types is the time scale.

For any BD OBS assay application, we typically recommend a simple proliferation experiment such as the preceding as a first experiment. This serves two purposes: it allows the user to gain familiarity with the collection and processing of BD OBS data, and it gives an indication as to a suitable number of cells to use for the assay in mind. In the case of the CHO cell data shown in *Figure 1*, for instance, the number of cells to seed at the beginning of the assay might depend upon how long the assay was expected to run and when the readings might be taken. Thus, it would make no sense for a 12-hour assay to seed only 1600 cells, as there would be no signal until 100 hours. Conversely, for a five-day assay, it would make no sense to seed 100,000 cells per well, as by day five they are dying and decreasing in number.

## Doubling Time Analysis

To perform a doubling time analysis, it is not necessary to know the exact number of cells seeded, only their relative concentrations across the dilutions. If the actual cell numbers are known, however, the doubling time curve becomes fully quantitative and can serve as a calibration curve. It is then possible from the amount of time it takes an unknown sample to come positive to deduce its starting density.

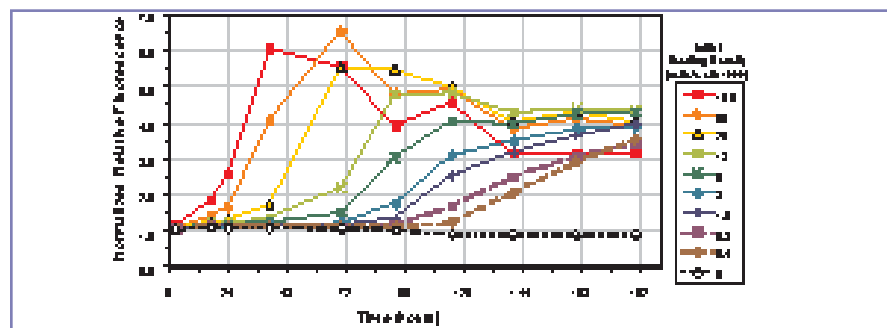
As the first example, consider the CHO growth curves shown in *Figure 1*. Since we have shown that normalized relative fluorescence (NRF) corresponds to cell number, we know that any given value of NRF corresponds to a given cell density (absent any other factors).<sup>1</sup> To determine the doubling time, simply note the time it takes each seed density to reach a given "threshold" value of NRF. It may be necessary to interpolate to do this. We recommend picking a threshold value intermediate between the baseline and the inflection point on the curve. The actual value is arbitrary, so pick one that most of your curves reach. For this example, we chose NRF = 2.

Simply construct a plot where the time to the threshold NRF is plotted against the seed density. Such a plot is shown in *Figure 2* for the CHO proliferation data shown in *Figure 1*. The slope of a logarithmic fit is proportional to the doubling time, which may be obtained by multiplying the obtained slope by the log of two (use either natural log or common log, depending on what was used to fit the data).

Therefore, if you plot along the x-axis the log-base-2 of the seed density, the slope of a linear fit to the data will be the doubling time directly. Such a plot of the same CHO data is shown in *Figure 3*. This analysis requires an extra calculation up front, but the slope directly gives you the doubling time. To calculate log-base-2, use the following formula:

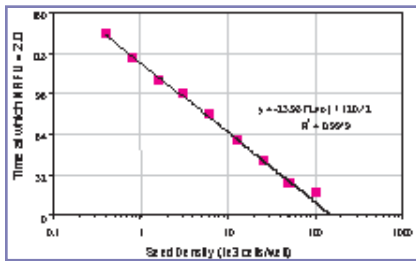
$$\log_2(x) = \log_{10}(x) / \log_{10}(2) = \ln(x) / \ln(2).$$

*continued*

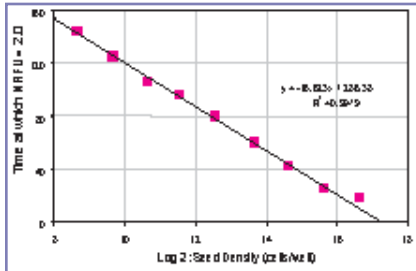


**Figure 1:** Actual normalized BD OBS data over time for various seed densities of CHO cells. Cells were cultured in suspension in BD OBS wells in 200  $\mu$ L of media at 37°C, 5% CO<sub>2</sub>.

## Doubling Time Analysis (continued)



**Figure 2:** Growth kinetics plot to determine doubling time. X-axis is actual seed density shown on a log scale. To convert slope to the doubling time, multiply by the natural log of two,  $\ln(2)$ .



**Figure 3:** Growth kinetics plot to determine doubling time. Here, the X-axis is the log-base-2 of the actual seed density. The slope of such a plot equals the doubling time directly.

## Same Approach Holds for Microbes

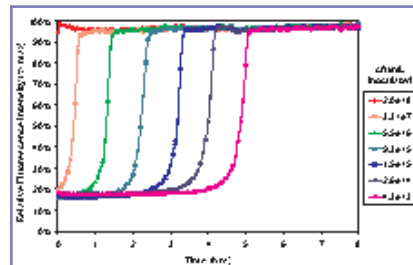
Such analyses have been done for a variety of mammalian cell types, as well as for yeast and bacteria. **Figure 4** shows a series of proliferation curves for serially diluted inocula of *E. coli*. **Figure 5** shows the corresponding doubling time plot. Other than the vastly different time-scale, it can be seen that these plots are essentially identical to those derived for CHO cells.

The doubling time plot also serves as a calibration curve, which allows you to ascertain the number of cells that were seeded into a well if you know how long that well took to reach the threshold NRF. This is particularly useful for bacteria and is how we recommend enumerating microbial samples.

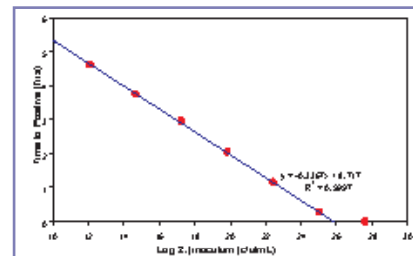
In addition to the slope, the other constants in the least-squares equation

have meanings. In the log-base-2 approach, the Y-intercept ( $x=0$ ) is the time required for a single organism to reach the threshold NRF, since the log-base-2 of 1 is zero. The X-intercept ( $y=0$ ) is the number of organisms that will give the threshold signal at time 0.

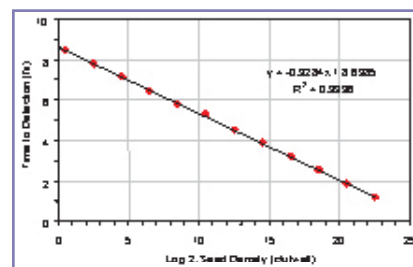
That this correlation between "time to detection" and seed density is highly linear and holds across a broad range of seeding densities can be seen in **Figure 6**, which shows such a plot for a strain of salmonella serially diluted to the extent that there was no more than a single bug in each well. Consistent with the preceding interpretation of the Y-intercept, this curve shows that a single organism came positive in 8.6 hours.



**Figure 4:** Actual normalized BD OBS data over time for various seed densities of *E. coli*.



**Figure 5:** Growth kinetics plot to determine doubling time, *E. coli* example.



**Figure 6:** Growth kinetics plot for salmonella. Doubling time was 0.33 hours, and the time for a single organism to come positive was 8.6 hours.

## How To Use This Kind of Information

### Proliferation/Media Optimization Studies

The rate at which cells proliferate is a function of the conditions under which they are grown, including the composition of the media in which or the surface upon which they are cultured. Using the growth kinetics protocol outlined herein, it is simple and straightforward to quantitatively assess the impact on growth of the culture conditions. Such a technique can be used to perform high-throughput experiments to optimize growth conditions for a given cell type or to investigate the impact of a series of drugs on proliferation.

### Assess Mutant Prevention Concentration of Antimicrobials

Resistance of microbes to antibiotics is an increasingly large concern. It is characterized by having a small fraction of a population survive treatment with a drug, either because a naturally occurring mutant was present or because the drug induces a mutation. If a small fraction of an inoculated culture survives treatment with drug, it will eventually come positive. The time it takes to come positive will allow you to determine the effective starting density, from which you can estimate the fraction which survived the drug. Because the BD™ Oxygen Biosensor System allows such analysis non-destructively, it would then be possible to isolate the surviving organisms from such wells for further study (e.g., to confirm their resistance).

### Reference

1. Wodnicka, M., et al., *JBS* 5:141 (2000).

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