

# Microbial Applications of the BD™ Oxygen Biosensor System

- As with other cell-based applications, we recommend as a first experiment a simple proliferation assay utilizing a range of seeding densities to identify suitable conditions for the assay of interest (*Technical Bulletin #438*). Perform a dilution series of the organism of interest and follow cell growth over time. Since temperature, pH and other culture variables such as media composition can impact growth kinetics, this preliminary experiment should ideally be run at the same conditions as the assay you wish to develop. Read at time intervals appropriate for the organism of interest (e.g., every 5-30 minutes is recommended for bacteria because they quickly double). This will yield a series of sigmoidal growth curves. The lower flank of the sigmoidal curve represents cell densities below the detection threshold; the upper flank represents cell densities above that which reduces oxygen to zero in the sensor layer. Based on the anticipated duration of your assay, and whether you are looking for an increase or decrease in oxygen consumption, you should be able to deduce from these data a suitable seeding density.

- There is approximately one order of magnitude of dynamic range in the signal, so for cells which multiply rapidly, such as bacteria, we recommend quantitating on the basis of how long it takes for a well to demonstrate an increase in signal rather than on signal magnitude. A plot of time-to-detection vs. log of seed density yields a straight line plot, the slope of which correlates to doubling time. If log-base-2 of the seeding density is used as the x-axis variable, the slope will equal the doubling time (*Technical Bulletin #438*).
- The BD™ Oxygen Biosensor System is amenable to single-time-point as well as kinetic assays for antibiotic susceptibility/resistance testing. Each approach offers specific advantages.
  - Understanding the baseline growth kinetics in the absence of drug (obtained via the preliminary experiment described herein) should provide insight into a suitable timeframe during which a single reading may be taken. If the goal is to minimize the assay time, for instance, choose a seed density for which a positive signal is rapidly obtained. You could then look shortly after this time for wells that fail to come positive in a short-duration assay as a quick and rapid screen for antibiotic activity. The advantage here is a rapid, single-point assay. The limitation is that you will have no ability to detect wells that come positive at much later times, which could indicate a low level of resistance.
  - Kinetics assays can potentially provide insight into the mode of action of the antibiotic. For instance, some drugs may delay the onset of growth, whereas others may alter the shape of the growth curve. The advantage of kinetic assays is the far greater richness of information they can yield.
- The magnitude of delay or the change in shape of a growth curve and its relationship to dose may be informative. For instance, since the time to positive relates to seeding density, the delay in growth due to the presence of drug represents the degree to which the drug effectively suppressed growth. The longer the delay, the lower the effective viable concentration. Such knowledge can potentially be used to identify and quantitate antibiotic resistance.
- The BD Oxygen Biosensor System was designed to afford flexibility in terms of work flow. There is no single "right way" to do these assays, so feel free to develop the protocol to suit your needs. Simply keep in mind the impact your workflow may have on the oxygen concentration in the media so that you can unambiguously interpret the readout.

For additional information on the BD™ Oxygen Biosensor System, visit our website at:

[www.bdbiosciences.com/discovery\\_labware/Products/drug\\_discovery/oxygen\\_biosensor\\_system](http://www.bdbiosciences.com/discovery_labware/Products/drug_discovery/oxygen_biosensor_system)

## BD Biosciences Discovery Labware

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