



## Guidelines for the Use of BD Oxygen Biosensor Systems

BD Oxygen Biosensor Systems are intended for a variety of cell-based and subcellular screening assays for the drug discovery market. Potential applications for this product include assays where measuring changes in dissolved oxygen concentration in real time is of interest. Examples of such assays include: viability, proliferation, and growth kinetics experiments for prokaryotic or eukaryotic cells; toxicity screening; antibiotic susceptibility testing; oxidative metabolic screening; etc.

1. Optimal fluorescence parameters: ~485 nm excitation and ~630 nm emission, although there is considerable bandwidth surrounding each wavelength.
2. We recommend the following quick and easy non-biological control experiments to verify that your instrument settings are appropriate:
  - a) Read an empty Oxygen Biosensor System (as received) side-by-side with an empty BD Falcon™ Multiwell plate of the same format. The average fluorescence signal from the Oxygen Biosensor System should be at least 10-fold greater than that of non-sensor BD Falcon™ plate.
  - b) When performing actual assays, run 100 mM sodium sulfite in at least one well as a positive control, and plain media alone in at least one well. The normalized signal (see paragraph 6 below) due to the sulfite should be at least 5-fold greater than that of the plain media.
3. Ideally, the plates should be read from the bottom, thereby negating any potential scattering issues associated with reading from above the plate.
4. Within a given assay, the system may be read as many times as you would like.
5. For systems based upon a 96-well format, 200 µl is a typical per-well volume.
6. We advise pre-blanking the entire plate, so that each well may be referenced against its own initial signal. We then typically normalize signal for each well at any given time point to that for the same well at the start of the experiment, and express the signal as “normalized relative fluorescence units.” Expressed in this fashion, the numbers then represent a “fold” increase over the no-cell control.
7. As a starting point, we recommend at least 10<sup>6</sup> cfu/mL of bacteria or 50,000 mammalian cells/well, but you will need to determine what is optimal for your particular application. For evaluation purposes, use as many cells as reasonably possible to minimize the time required for signal to appear, or seed a range of cell numbers and follow the signal over time to ascertain suitable starting conditions for a particular assay. Oxygen Biosensor Systems work with a variety of cell types, including bacteria, fungi, multicellular organisms, and immortalized mammalian cell lines. However, because the growth surface has not been tissue culture treated, the systems may not be suitable for use with anchorage-dependent cell lines. For applications requiring adherent cells, please contact our Technical Support group.
8. The sensor technology is very flexible, which means the systems support a number of work flows - you can add the cells and the drug in any relative order. Hence, you can either establish good growth (high signal) and then kill off the cells with drug and follow the decrease in signal relative to no-drug controls, or you can seed the cells with the drug and watch for the absence of growth (increase in signal) relative to no-drug controls.
9. The rate at which cells consume oxygen can depend heavily upon temperature. Be sure to consider such effects when designing the work flow for a particular assay.
10. It should be noted that the “baseline” fluorescence of an empty plate will differ slightly from that of a plate filled with media or one filled with media and equilibrated in a 37°C, 5% CO<sub>2</sub> incubator, due to slight changes in the equilibrium oxygen concentration in the vicinity the dye. For most applications, this effect is insignificant relative to the large increase in signal once the cells begin to grow, and should have no effect on your ability to draw conclusions.
11. Remember - ***“when it grows, it glows”***.
12. For research use only, not for use in diagnostic procedures.