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BD™ Oxygen Biosensor System vs. Existing Methods of Measuring Toxicology

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BD™ Oxygen Biosensor System

The BD™ Oxygen Biosensor System (BD™ OBS) is a fluorescence-based method that measures the amount of oxygen consumed over time. The consumption of oxygen is quantifiable if desired. The amount of oxygen consumed is based on the rate of cell respiration or mitochondrial activity. The BD OBS is read on common fluorescent plate readers and it does not interfere with cell growth.

Assay: Every cell type would need to be titrated on the BD OBS to determine the appropriate seeding density depending on the type and length of time for the experiment planned.

Advantages

1. Rapid: measure by a standard bottom read fluorometer (approximately one minute/plate depending on brand).
2. Kinetic assay: multiple reads can be taken for each plate over a prolonged length of time.
3. Homogeneous: system for measuring oxygen consumption or cell death is contained within the plate. No solutions or other chemicals are required and thus, there is no potential interference with drug candidates.
4. Automatable.
5. Non-toxic to all cells tested.
6. Reversible: measure the growth of cells, toxicity and recovery of cells and also determine the difference between a drug that is cytostatic and cytotoxic.
7. Can be multiplexed with other fluorescent assays.

Disadvantages

1. Requires a fairly large number of cells, and is currently available for non-adherent cells.
2. Temperature sensitive.
3. Potential for interference from drugs that fluoresce in the 590-630 nm range.

Alamar Blue

Alamar Blue is thought to behave similarly to MTT although much less is known about its action. It is believed to be converted to a pink product by a mitochondrial dehydrogenase although this is not absolutely known. The assay can be used for short end point assays or over longer periods of time. The company that makes it suggests that alamar blue be added to the start of a study and analyzed in a kinetic fashion over a 48-hour period.

Assay: Cells to be standardized relative to seeding density and for time needed to convert alamar blue to its pink product.

Advantages

1. Use with a small number of cells over an extended period of time because it converts the alamar blue slowly (dependent on cell number).
2. Use as an endpoint or kinetic assay.
3. Soluble and is released by the cells; does not require solubilization.
4. Absorbance or fluorescence read.

Disadvantages

1. Product formation is irreversible. In performing a long-term assay you may get false positives if cell converts to pink product but subsequently dies. Timing of alamar blue addition may be critical.
 - BD OBS is reversible and can be measured over time.
2. Alamar blue could potentially interfere with a drug candidate since both are present with the cells.
 - BD OBS does not interfere with any drug candidate.
3. Length of time it takes a particular cell type to convert alamar blue to product, is dependent on cell type, number of cells, when the alamar blue is added and for what length of time it is to be incubated.
 - BD OBS signal is dependent on cell type and number of cells. Optimal signal needs to be determined for cell type, but is independent of any drug addition other than the drug candidate.

MTT

The MTT colorimetric assay determines the ability of viable cells to convert soluble tetrazolium salt [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) into an insoluble precipitate. MTT is reduced at sites in the mitochondrial electron transport system and is a test for succinate dehydrogenase activity. The reaction converts yellow salts to blue crystals that are dissolved and read spectrophotometrically.

Assay: Has to be set up for each cell line with regards to optimal seeding density of the cells, the duration of experiment and the time of MTT incubation.

Advantages

1. Considered a major advance; is the most prevalent *in vitro* assay used.
2. Rapid, versatile, quantitative and highly reproducible (+/- 15% SD).
3. Adaptable to large-scale screening; relevant for most cells.
4. MTT reduction correlates to indices of cellular protein and viable cell number.
5. More sensitive and earlier predictor of toxicity than classical LDH or neutral red measurements.

Disadvantages

1. There are cell lines that do not metabolize the MTT well or have an acceptable colorimetric profile for control cells (no drug).
 - BD OBS does not have this limitation.
2. Production of the MTT product is dependent on the MTT concentration in the culture medium. The kinetics and degree of saturation are dependent on cell type.
 - BD OBS does not have this limitation.

continued

MTT (continued)

3. Assay is less effective in the absence of cell proliferation.
 - BD OBS does not have this limitation, given a sufficient seeding density.
4. The presence of glutathione-S-transferase (a normal enzyme that protects cells) can reduce the MTT independent of toxicity. These cells give high background and potentially false positives as stated in a recent paper.
 - BD™ OBS does not have this issue or concern.
5. MTT cannot distinguish between cytostatic and cytotoxic effect.
 - BD OBS is able to differentiate because (1) the system can be used over time to determine whether cell death occurs, or (2) the drug can be removed (either by operator or by metabolic process of cell) and cells can resume growth which can be measured.
6. Individual cell numbers are not quantitated and results are expressed as a percentage of control absorbance.
 - BD OBS: when correct controls are performed, the fluorescence intensity correlates to cell number, which must be established for each cell type.
7. Test is less effective if cells have been cultured in the same media that has supported growth for a few days, which leads to underestimation of control and untreated samples.
 - BD OBS does not have this limitation. Typically the cells are plated into the BD OBS +/- drug and measured over time. Cell proliferation can be measured in the absence of media change for up to five days.

8. Certain types of drugs (i.e. interferon) can induce formazan production (MTT) and/or mitochondrial activity. Increased production of formazan will potentially give false positives with these drugs.
 - BD OBS does not depend on the production of formazan, therefore, this error will not occur. The induction of mitochondria or mitochondrial respiration can be measured by the BD OBS which may look like an increase in growth. However, since multiple time points can be measured, any potential cell death would be apparent.

LDH

Lactate dehydrogenase (LDH) activity in the supernatant of cells can be used to determine cell membrane damage. This is indicated by the leakage of the LDH out of cells into the medium. This is the preferred method to measure cell damage. The extent of cell damage is then correlated to cell death.

Assay: Cells need to be standardized to cell number and technique used to measure LDH. The higher number of cells correlates to a higher enzyme value. Assay is an end point assay.

Advantages

1. Can differentiate between cytostatic and cytotoxic since it is dependent on membrane disruption.
2. Fast, inexpensive (measure absorption at 340 nm).
3. Measures cell damage.

Disadvantages

1. The drug or particulate matter may affect the LDH activity.
 - BD OBS does not have this limitation.
2. The serum used in the cell culture medium must be treated to remove endogenous LDH activity.
 - BD OBS does not have this limitation.

3. Serum may, to some extent, exert an inhibitory effect on the enzyme assay. (Appropriate controls would allow for this eventuality).
 - BD OBS does not have this limitation.
4. Cells can be left for up to three hours in LDH assay without markedly influencing results. (Assay is stable for approximately three hours although small changes may occur).
 - BD OBS is measured in real time and not as an end point. There is no biochemistry for which stability is an issue.
5. Prior to instituting this test, it would have to be demonstrated that LDH activity for a particular cell type correlates well to cell death. This is not true for all cell types.
 - BD OBS does not have this limitation.
6. LDH is not as sensitive as MTT.
 - BD OBS is as or more sensitive than MTT.
7. LDH does not correlate to cell number.
 - BD OBS signal correlates to cell number.
8. Since there is a biochemical step, a cell manipulation step and requires numerous controls, the assay is less high throughput.
 - BD OBS requires no additional manipulations. Moreover, if the researcher wanted to specifically measure cell damage as a parameter in his study, he could use cells from the BD OBS. Therefore, derive cell death, if any, directly from BD OBS and cell damage from the secondary LDH assay.

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