

- **Easy-to-Use**
- **From 100-Fold to 600-Fold Enhancement**
- **Requires Fewer Cells**
- **Responds to Any Oxidase Stimulant**
- **Non-Cytotoxic**

National Diagnostics' Diogenes Cellular Luminescence Enhancement System is a superoxide chemiluminescent enhancer that is non-denaturing to living cells. Superoxide radical (O_2^-) is produced intracellularly as a consequence of aerobic metabolism and extracellularly by leukocytes in response to infection. The extent of "oxidative burst" produced by white blood cells (WBCs) when stimulated by f-met-leu-phe, phorbol esters, anti-Fc receptor antibodies or LPS is a partial indicator of the immunocompetence of the cells tested. Currently, the production of O_2^- by leukocytes is monitored by such cumbersome and indirect methods as measuring oxygen uptake in a Clark electrode (both in the presence and absence of cyanide) or measuring spectral changes caused by the reduction of cytochrome c. As a non-cytotoxic intermediate in the mechanism of photon production, Diogenes is ideally suited to the detection of cell-mediated superoxide production. The intensity of light produced by Diogenes in the presence of superoxide is directly proportional to the O_2^- concentration, but is much higher than that achieved by using luminol. Therefore, Diogenes is ideal for monitoring cellular immunocompetence, utilizing a luminometer to quantify the light output. Any stimulant that activates an oxidase to produce extracellular superoxide is usable with Diogenes. Such means can be physiological or mimetic of the physiologic pathway.

Diogenes Improves Existing Technology

Traditional methods of detecting superoxide production include (1) the reduction of exogenously supplied cytochrome c; (2) measurement of oxygen uptake using a Clark electrode; and (3) luminol-mediated chemiluminescence. With a signal range spanning over three orders of magnitude, luminol-mediated chemiluminescence offers the greatest sensitivity, is the most accessible and requires the least number of cells. However, until now, chemiluminescence signal output has been limited. Diogenes overcomes this handicap by increasing the superoxide-to-photon release ratio.

Diogenes Is Specific for Superoxide

The response of the Diogenes Cellular Luminescence Enhancement System to superoxide is at least 10^6 greater than that for hydrogen peroxide (H_2O_2). This means that peroxide formed by dismutation of O_2^- will not interfere in the assay. Backgrounds are kept low and results may be interpreted with confidence.

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Diogenes Enhances Sensitivity

The Diogenes Cellular Luminescence Enhancement System enhances the sensitivity of the chemiluminescence assay to more accurately detect lower concentrations of superoxide anion. It is estimated that under non-enhanced conditions, only one (1) photon is released for each one thousand (1000) molecules of superoxide produced. By substantially increasing the photon release ratio, Diogenes improves sensitivity by at least two (2) orders of magnitude. This means that Diogenes is capable of detecting at least 100-fold lower concentrations of superoxide. This translates to fewer needed cells and greater monitoring ability of targeted cells.

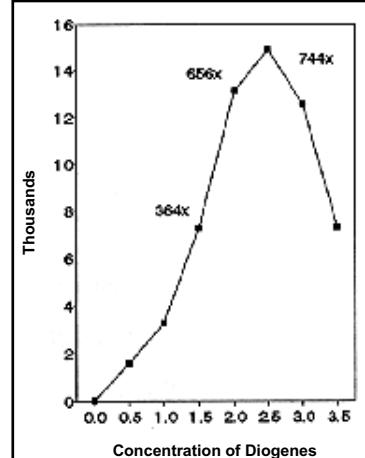
Diogenes Maintains Cell Sample Integrity

The Diogenes Cellular Luminescence Enhancement System is non-toxic and non-denaturing to living cells and does not interfere with the normal cellular response. Diogenes is highly selective for superoxide and does not induce up-regulation of superoxide production or any alterations of cellular pathways. In the absence of superoxide, Diogenes will not cause luminol cleavage. Therefore, there is virtually no background, and Diogenes itself does not stimulate superoxide production.

Diogenes Enhances Signal Output

The Diogenes Cellular Luminescence Enhancement System contains the luminescent compound luminol plus the enhancer complex of luminol. Diogenes has been shown to routinely increase chemiluminescent millivolt (mV) output from 100-fold to greater than 600-fold.

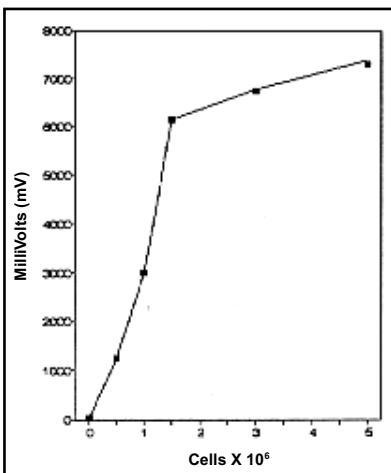
Enhanced Cellular Chemiluminescence



At optimum concentration, Diogenes can increase output by greater than 600-fold.

A minimum number of cells are required for the Diogenes Cellular Luminescence Enhancement System. In fact, increasing cell concentrations over the suggested number of cells has only limited effect on the sensitivity of the assay.

Fewer Cells Are Needed



Increased cell concentrations yield only a limited increase in sensitivity. Inhibition at higher concentrations is due to a self-quenching by cells absorbing emitted photons.

Diogenes Detects Response to Multiple Stimuli

The Diogenes Cellular Luminescence Enhancement System will respond to any extracellular superoxide regardless of the means by which the cells are stimulated. Any signal that activates an oxidase enzyme complex will result in enhanced chemiluminescence when Diogenes is present. The stimulatory signals may be either physiologic or mimetic of the physiologic pathway. Known stimulants of superoxide production include any ligand that cross-links Fc receptors (e.g. mAb197), phorbol myrisate acetate (PMA), and f-met-leu-phe (fMLP).

Diogenes may be used to assay Superoxide Dismutase (SOD), which is routinely assayed using a standardized O_2^- -generating system (usually xanthine/xanthine oxidase) coupled to a superoxide detection system (McCord & Fridovich, *J.Biol.Chem.* **244**:6049, 1969). Diogenes can be substituted for any superoxide detection system with a resulting increase in sensitivity. This will translate to less O_2^- needed per assay and thus a greater sensitivity for SOD detection. Typically, 10^{-5} units or less of Xanthine Oxidase (XO) in 0.5 mM Xanthine gives a convenient baseline signal of O_2^- , depending upon the sensitivity of the luminometer utilized.

DIOGENES KIT

Order No. CL-202

1 Kit

For Ordering or Additional Information Contact National Diagnostics:

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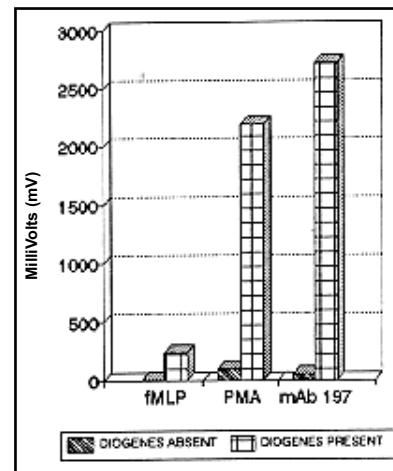
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Responds to All Stimulants



Diogenes will detect superoxide regardless of the source.

Diogenes is Easy-to-Use

The Diogenes Cellular Luminescence Enhancement System is easy-to-use and requires only a luminometer and no lengthy laboratory procedures. The Diogenes Kit is a two-component system consisting of the **Diogenes Reagent** and the **Diogenes Activator**.

SOLUTION PREPARATION

1. Add 1.0ml of deionized water to the Diogenes Reagent that is contained in the vial. Mix until **completely** dissolved.
2. Add 9.0ml of deionized water to the Diogenes Activator that is contained in the bottle. Mix until **completely** dissolved.
3. Add the contents in the vial (Diogenes Reagent) to the contents in the bottle (Diogenes Activator), recap the bottle and shake vigorously to mix.
4. The combined solution (10ml) comprises the Diogenes Complete Enhancer in its ready-to-use, final working strength, and will yield up to 100 assays.

CELLULAR ASSAY

1. Add 5×10^4 - 5×10^5 cells contained in glucose media into a luminometer cuvette or microtiter plate.
2. Add 100 μ l of Diogenes Complete Enhancer Solution.
3. Add 20 μ l of stimulant (PMA, mAb197, fMLP, etc.).
4. Read the results. Time to peak output will depend on the cell and stimulant used. The response of Diogenes to superoxide is instantaneous.

STORAGE OF DIOGENES

1. The prepared Diogenes Complete Enhancer Solution can be stored at 4-8°C for up to 60 days.
2. The shelf-life of the non-reconstituted reagents in original packaging is one (1) year.