SCREENING REACTIVE OXYGEN SPECIES (ROS) ON IQUE® SCREENER

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Total ROS/Superoxide detection kit (ENZ-51010)

SUMMARY

The iQue Screener was used to simultaneously detect levels of ROS and superoxide in multiple cell lines.

- Both Total ROS (non-superoxide) and superoxide were detected simultaneously.
- Two related endpoints were easily discriminated by proper indicator selections and confirmed by differential inhibition.
- Easy to use ForeCyt provides a friendly screening-centric interface to create assay protocols and analyze results.

PROBLEM

Reactive oxygen species (ROS) is a group of highly unstable molecules including H_2O_2, NO, and O_2- that are generated in situ from various stressors. At least one ROS molecule (NO) acts as signaling molecule, migrating across proximal cell membranes to activate guanylyl cyclase and cause smooth muscle relaxation. At higher concentrations ROS cause oxidative stress and are destructive to lipids, DNA and proteins. Excessive amounts have been linked with numerous diseases such as cancer, cardiovascular disease and hearing loss. General aging effects are also implanted to be the result of ROS. There are many control mechanisms in play to limit the damage ROS would otherwise cause, including enzymes and vitamins. Early, rapid and easy identification of compounds that cause increases in ROS would be a valuable component to a drug discovery and development program.

RESULTS

Total ROS and superoxide were simultaneously measured in two different cell lines using the Total ROS/Superoxide detection kit (ENZ-51010) from Enzo Life Sciences. T-cell derived Jurkat cells and HeLa cells both have previously been shown to produce ROS in abundance following stimulation with pyocyanin. Both cell lines were treated with pyocyanin to stimulate ROS and superoxide generation, and with N-acetyl-cysteine (NAC) to inhibit ROS. Five mM NAC inhibited about 90% of Total ROS in Jurkat cells treated with 100µM pyocyanin (Figure 1) and 50% in HeLa cells, but only about 20% of superoxide in Jurkat cells and 10% in HeLa cells. This differential inhibition supports the notion that what is detected by the 2 dyes is different.

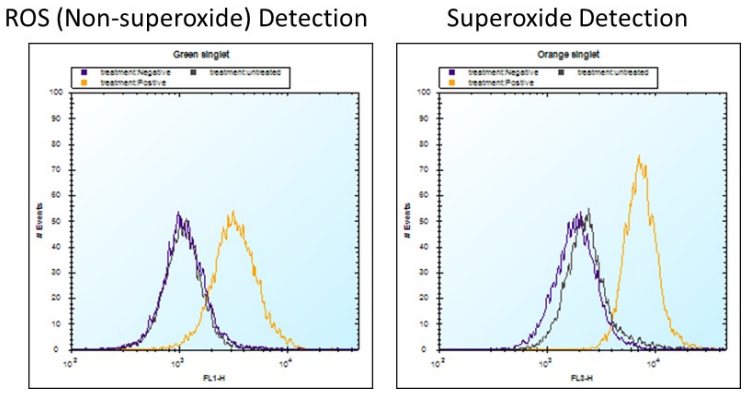

Figure 1 Histogram overlay of control (black), activated (orange), and inhibitor pre-treated (purple) Jurkat cells used in ROS detection. Treated cells were activated for 30 minutes with 200µM pyocyanin after 30 minutes of none or 5mM N-acetyl-cysteine (NAC) pretreatment. Dyes were added after treatments 30 minutes before reading on iQue. HeLa cells responded similarly to the Jurkat cells when treatments were carried out in the presence of Accutase.

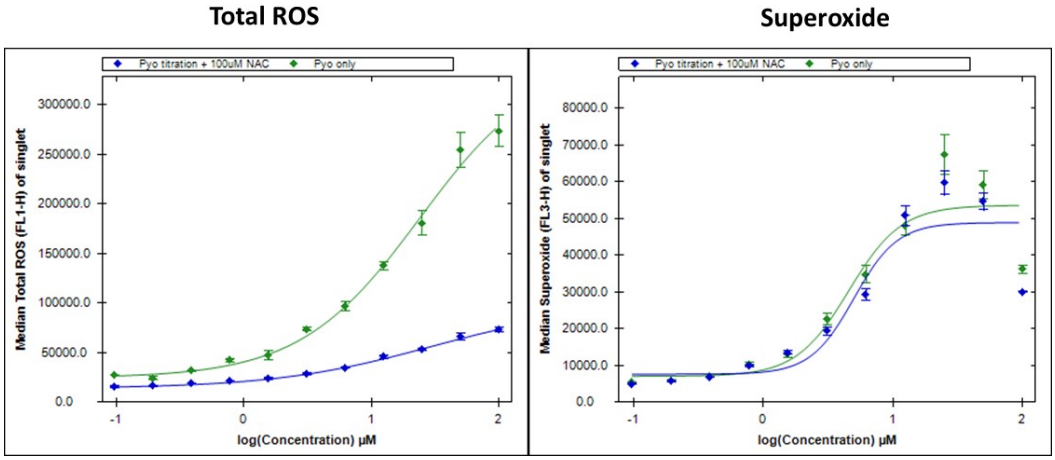

Figure 2: Dose responses of pyocyanin in generating Total ROS or superoxide in HeLa cells, with or without pretreatment with 5mM NAC. Total ROS signal is mostly inhibited by 5mM NAC, whereas superoxide is not. Data are based on triplicate +/- S.D.

THE INTELLICYT SOLUTION

The iQue analyzes individual cells for 6 parameters. In this application, every sampled cell is analyzed for Total ROS and for superoxide, leaving 2 fluorescent channels unused. When combined with immunophenotyping, a mixed population like PBMCs could be treated and analyzed for differential alterations to their oxidative states. Questions involving immunophenotyping can be answered, for example: do T_H cells generate more total ROS than Tregs, when treated with LPS?

The iQue enables assay miniaturization. Typically the iQue samples about 2µl from each well, based on a 1 second sip time. This time can be reduced to take as little as 1µl, meaning very small assay volumes can be used. We routinely run 6µl assays in 1536-well plates with the iQue HD and 10-30µl assays are easy in 384-well plates on the standard iQue. This means using fewer cells in each analysis, opening the door to using primary or stem cells for ROS/SO screening.