Screening Reactive Oxygen Species (ROS) on the iQue Screener

SUMMARY

The iQue 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 implicated 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 from Enzo Life Sciences. Jurkat cells are T-cell derived and a work horse in our labs. The infamous HeLa cells are ubiquitous in biotech and had 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 100 µM 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 suggestion that what is detected by the 2 dyes is different.

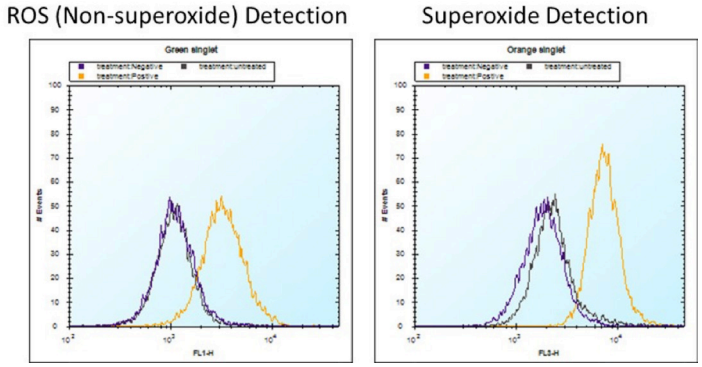

Figure 1. Histogram overlay of control (black), activated (orange), and inhibitor pre-treated (purple) Jurkat cells used in ROS detection. Treated cells were activated for 30 minutes with 200 µM pyocyanin after 30 minutes of none or 5 mM N-acetyl-cysteine (NAC) pretreatment. Dyes were added after treatments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

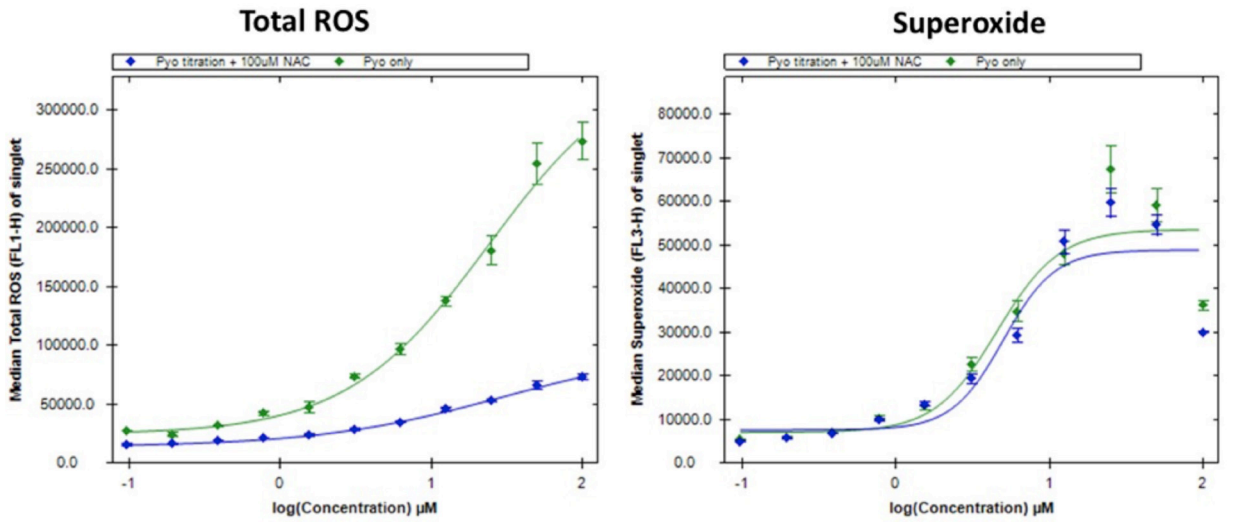

Figure 2. Dose responses of pyocanin in generating Total ROS or superoxide in HeLa cells, with or without pretreatment with 5 mM NAC. Total ROS signal is mostly inhibited by 5 mM NAC, whereas superoxide is not. Data are based on triplicate ± S.D.

THE INTELLICYT SOLUTION

- **The iQue analyzes individual cells for 6 parameters** – Individual objects are analyzed at up to 10,000 per second. This high rate of data acquisition contributes to the rapid screening rate and robust results the iQue delivers.
- **The iQue enables assay miniaturization** - Typically the iQue samples about 2 µL from each well, based on a 1 second sip time. This time can be reduced to take as little as 1 µL, meaning very small assay volumes can be used. We routinely run 6 µL assays in 1536-well plates with the iQue HD and 10-30 µL 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.

To learn more about how iQue can improve your screens, please visit **www.intellicyt.com**

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