Abstract: In order to identify selective, novel oxygen species (ROS) generating agents as a novel therapeutic strategy for the treatment of drug resistant cancer cells, we developed a live-cell microplate based assay for the rapid and quantitative assessment of ROS. The assay is based on the conversion of 2',7'-dichlorofluorescein diacetate (DCFDA) to 2',7'-dichlorofluorescein (DCF) by intracellular esterases. The assay can be performed with EX: 495 nm and EM: 530 nm. By using such a diacetate derivative, the assay can be performed with both adherent and non-adherent cell lines, upon treatment with the common chemotherapeutic drugs doxorubicin and idarubicin, and tert-butyl hydroperoxide (TBHP) (as a positive control) at concentrations between 20-100 µM for 30 min. Non-adherent cells were grown in culture flasks to a density of 6 x 10^5 cells/mL 24 hrs before the assays. The fluorescent signal was found to be 6-40 times control for cells treated with the drugs, while non-drug treated control cells showed a negligible signal at 20-100 µM. The fluorescence signal was also significantly decreased after treatment with antioxidants. The assay was optimized for use in high content screening using the BioTek Synergy Mx microplate reader, with a 40X magnification, 40 ms exposure time, and a FITC filter set. The assay was found to be highly reproducible with a precision of ±0.2 nm. The assay was found to be sensitive to ROS production from cell lines treated with the drugs, and was compared to negative and positive controls (no drug or tert-butyl hydroperoxide treatment). The assay was found to be highly reproducible with a precision of ±0.2 nm.

Methods:

- Development of a Live-Cell Based Reactive Oxygen Species (ROS) Assay for use in High-Content Screening of Drug Candidates Using The BioTek Synergy Mx Microplate Reader

**Results**

- **Results:**
  - The assay was found to be highly reproducible with a precision of ±0.2 nm.
  - The assay was found to be sensitive to ROS production from cell lines treated with the drugs, and was compared to negative and positive controls (no drug or tert-butyl hydroperoxide treatment).
  - The assay was found to be highly reproducible with a precision of ±0.2 nm.

**Discussion and Conclusions**

- The assay was found to be highly reproducible with a precision of ±0.2 nm.
- The assay was found to be sensitive to ROS production from cell lines treated with the drugs, and was compared to negative and positive controls (no drug or tert-butyl hydroperoxide treatment).
- The assay was found to be highly reproducible with a precision of ±0.2 nm.

**References**