

# A Live Cell, Kinetic, High Content Screening Application Interrogating Oxidative Stress in Neuroscreen-1™ Cells

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## Introduction

Oxidative stress, a natural by product of metabolism that damages proteins, lipids, and DNA when imbalanced, is studied in many areas of biomedical research, including aging, cancer, immunology, and several neurodegenerative diseases. High Content Screening (HCS) and the Cellomics® Oxidative Stress Reagent Kit allow researchers to investigate oxidative stress in an in-depth manner. Here, we examine oxidative stress in a subclone of PC-12 (rat pheochromocytoma) cells. These cells are exposed to various concentrations of two oxidative stress inducing agents: parasympathetic-mimetic tacrine<sup>1</sup> and the classic oxidant H<sub>2</sub>O<sub>2</sub>. The cells are then assessed by their intensity of a redox active dye.

## Materials and Methods

Neuroscreen-1 (NS-1) cells (a subclone of PC-12 cells; Thermo Fisher Scientific, Pittsburgh, PA) were plated and maintained on collagen I coated plates and flasks in RPMI media (15% serum, glutamine, nonessential amino acids, and antibiotics). The cells were plated in collagen I coated 96-well plates at a density of 4,000 cells/well and were grown in the presence of 200 g/mL nerve growth factor (NGF; BD Biosciences) to promote neurite outgrowth. The experiment was performed as a live cell assay where the cells were first stained for 30 minutes at 37° C with the Cellomics Oxidative Stress Reagent Kit, followed by compound exposure for 2 hours and subsequent fixation. The kit includes a blue DNA-binding stain to identify

nuclei and dihydroethidium (DHE), a dye that is oxidized into fluorescent ethidium by free radicals and intercalates into the cell's DNA. During the experiment, the cells were exposed to media alone or media containing 100 – 1,250 μM tacrine (Sigma) or 0.08% – 10% H<sub>2</sub>O<sub>2</sub> (Sigma) in duplicate. The Thermo Scientific Cellomics ArrayScan® VTI HCS Reader equipped with a Live Module (Thermo Fisher Scientific, Pittsburgh, PA) was utilized to take two images (blue to identify nuclei and red-orange for DHE) at 20x magnification every 20 minutes for two hours. The images were analyzed with the Cellomics Target Activation BioApplication to assess average DHE intensity as evidence of oxidative stress in each cell. A 20 minute incubation was used before imaging.

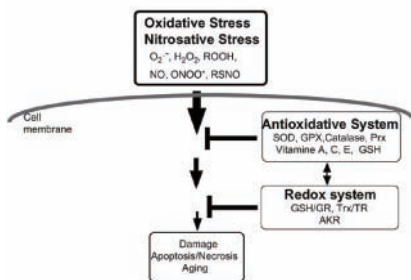


Figure 1. Diagram of Cellular Response to Oxidative Stress. Oxidative stress is a usual part of the environment for the average cell.

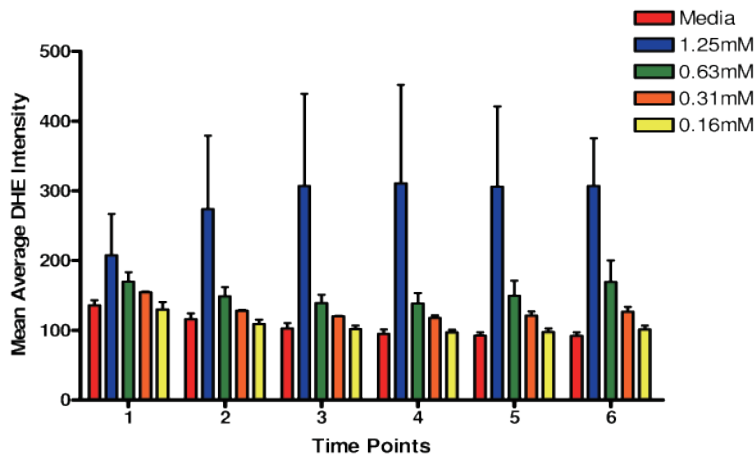


Figure 2. Time Course of Average DHE Intensity for Various Tacrine Doses. The graph illustrates the average intensity of DHE staining within the nuclei of cells treated with media alone or different concentrations of tacrine. A dose-dependent result can be seen at each time point, with the highest tacrine dose reaching its peak by the 4th time point. Each bar represents a condition in duplicate +/- SEM.

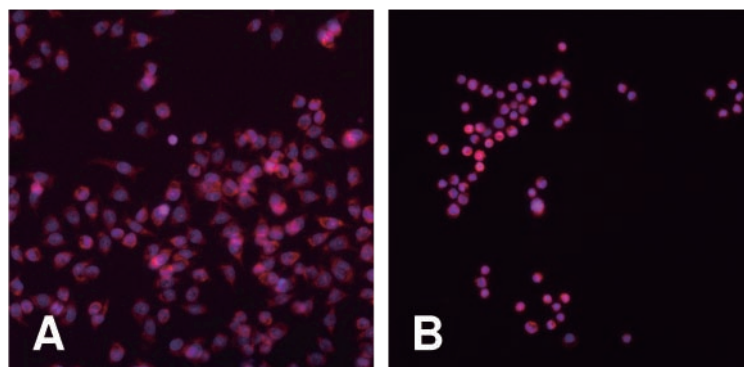
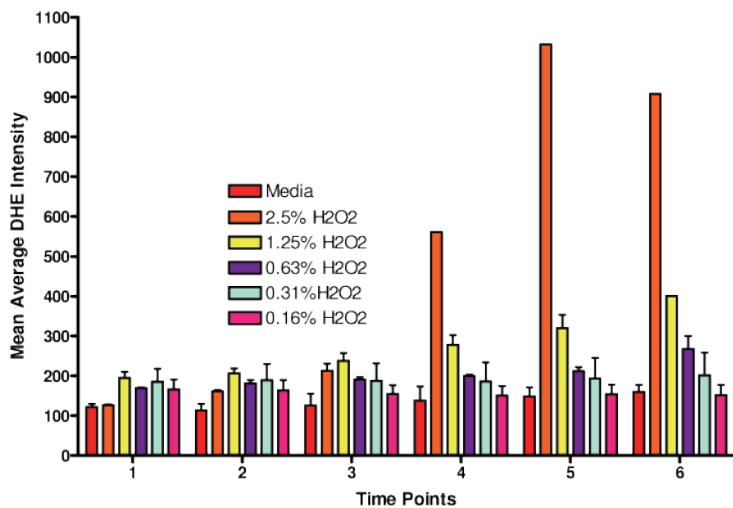


Figure 3. Images Acquired on the ArrayScan VTI HCS Reader. Composite 20x images of NS-1 cells treated with media alone (A) and 1.25mM tacrine (B) 80 minutes after exposure. The tacrine treated cells are smaller and rounder than their media alone counterparts and show a significant reduction in number of cells per well between the two treatments.



**Figure 4. Time Course of Well Average DHE Intensity for Various H<sub>2</sub>O<sub>2</sub> Doses.** The graph illustrates the average intensity of DHE staining within the nuclei of cells treated with media alone or different percentages of H<sub>2</sub>O<sub>2</sub>. The highest concentrations of peroxide (5 and 10%) resulted in wide spread cell death by time point 3 (data not shown). A dose-dependent result can be seen by the 4th time point, with the highest peroxide dose reaching its peak by the 5th time point and then declining. Each bar represents a condition in duplicate +/- SEM. Standard deviations cannot be reported in some treatments due to the acute toxicity in the replicates.

## Conclusions

- The Thermo Scientific Cellomics ArrayScan VTI HCS Reader, along with the Cellomics Oxidative Stress Reagent Kit permitted investigation of the kinetic response of oxidative stress in Neuroscreen-1 cells, a fast growing subclone of the PC-12 cell line. We have shown that when these cells are treated with known inducers of oxidative stress, a dose dependent and time resolved response is detected and measured. The tools used in this study permit:

- Out of the box, validated reagents used to detect the level of oxidative stress seen in multiple cell lines.
- A High Content approach to imaging and measuring the fluorescent oxidative stress response.
- A robust live cell assay permitting pharmacokinetic evaluation of toxic compounds.

The High Content Screening tools from Thermo Scientific demonstrate the best in class instrumentation, reagents, image processing and informatics to reduce the overall time to decision.

## References

- <sup>1</sup> Riekkinen M, Soininen H, Riekkinen P Sr, Kuikka J, Laakso M, Helkala EL, Partanen J, Riekkinen P Jr. "Tetrahydroaminoacridine improves the rency effect in Alzheimer's disease." *Neuroscience*. 1998 Mar;83(2):471-9
- <sup>2</sup> Diagram of Oxidative Stress Pathway <http://www.asiaandro.com/1008-682X/5/231.htm>

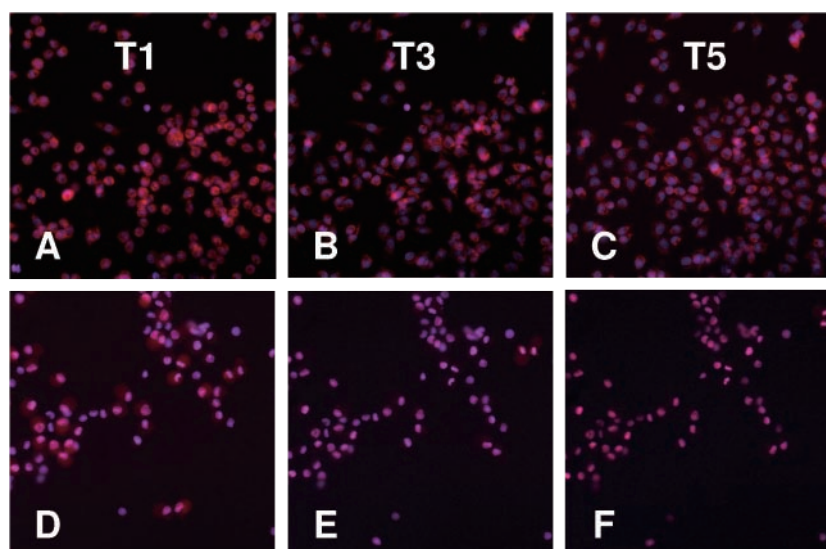
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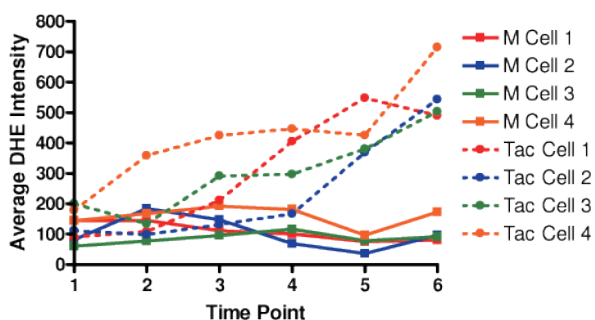
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**Figure 5. Images Acquired on the ArrayScan VTI HCS Reader.** Composite 20x images of NS-1 cells treated with media alone (A-C) or 1.25% H<sub>2</sub>O<sub>2</sub> (D-F) at time points 1, 3, and 5. The H<sub>2</sub>O<sub>2</sub> treated cells are observed as smaller and rounder than their media alone counterparts. H<sub>2</sub>O<sub>2</sub> produced bright DHE staining within the nucleus unlike the more disperse and dimmer DHE staining in the control cells.



**Figure 6. Cell-Level Comparisons of DHE Intensity on Individual Media Alone Cells Compared to 1.25mM Tacrine (Tac) Treated Cells.** The responses of four media treated cells (dotted lines) are compared to four tacrine treated cells (solid lines) over the time course of the experiment. The average DHE intensities for each of these cells were tracked and reported. The results demonstrate that individual media cells show low baseline DHE intensities over the time course, whereas tacrine treated cells showed increasing DHE intensities over the time course of the experiment. The results also show the dynamic response of cells in vitro when exposed to toxic insults.