# **ROS-Glo™ H<sub>2</sub>O<sub>2</sub> Assay - Novel Luminescence-Based Assay for ROS Measurement**

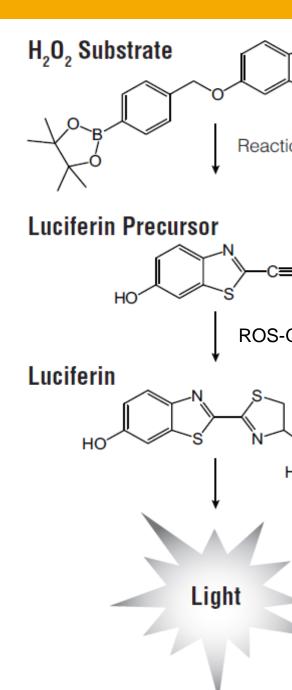
Gediminas Vidugiris<sup>1</sup>, Sarah Duellman<sup>1</sup>, John Shultz<sup>1</sup>, Jolanta Vidugiriene<sup>1</sup>, Hui Wang<sup>2</sup>, Jean Osterman<sup>2</sup>, Wenhui Zhou<sup>2</sup>, Poncho Meisenheimer<sup>2</sup> and James J. Cali<sup>1</sup> <sup>1</sup>Promega Corporation, 2800 Woods Hollow Rd, Madison, WI 53711; <sup>2</sup>Promega Biosciences LLC, 277 Granada Dr, San Luis Obispo, CA 93401

Abstract #257

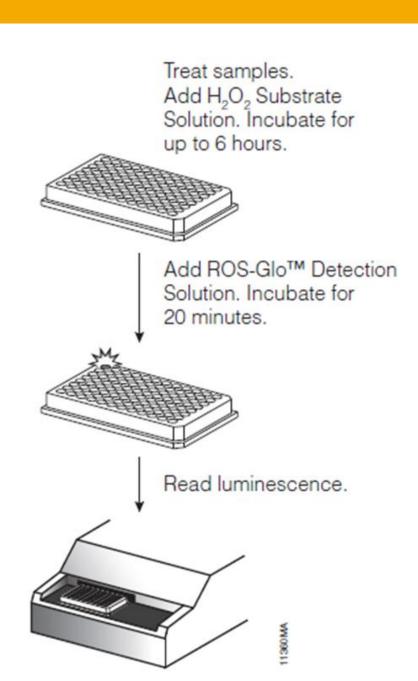
#### **1. Introduction**

 $H_2O_2$  is a reactive oxygen species [ROS] that is measured in cells as a marker of oxidative stress. It is also measured as a marker of enzyme activities that either consume or produce  $H_2O_2$ . It is desirable to screen chemical compounds for their capacity to alter  $H_2O_2$  levels in cultured cells or for their effects on  $H_2O_2$  levels in enzyme reactions. Current fluorescent assay formats are prone to false hit rates that are too high for efficient screening applications. We developed a novel luminescent  $H_2O_2$  assay (ROS-Glo<sup>TM</sup>) that detects  $H_2O_2$  directly, minimizes false hit rate and provides simple formats for cell-based and enzymatic assays.

Since various ROS are interconverted to  $H_2O_2$  in the cell and  $H_2O_2$ is the longest lived ROS, an increase in  $H_2O_2$  can reflect a general increase in the ROS level. Our method for measuring hydrogen peroxide utilizes the  $H_2O_2$  Substrate, which directly reacts with  $H_2O_2$ to produce a luciferin precursor. Addition of the ROS-Glo<sup>™</sup> detection solution converts the precursor to luciferin and provides luciferase and other components to produce a light signal proportional to the level of  $H_2O_2$ .



#### 2. Simple add-mix-read assay in cell culture wells

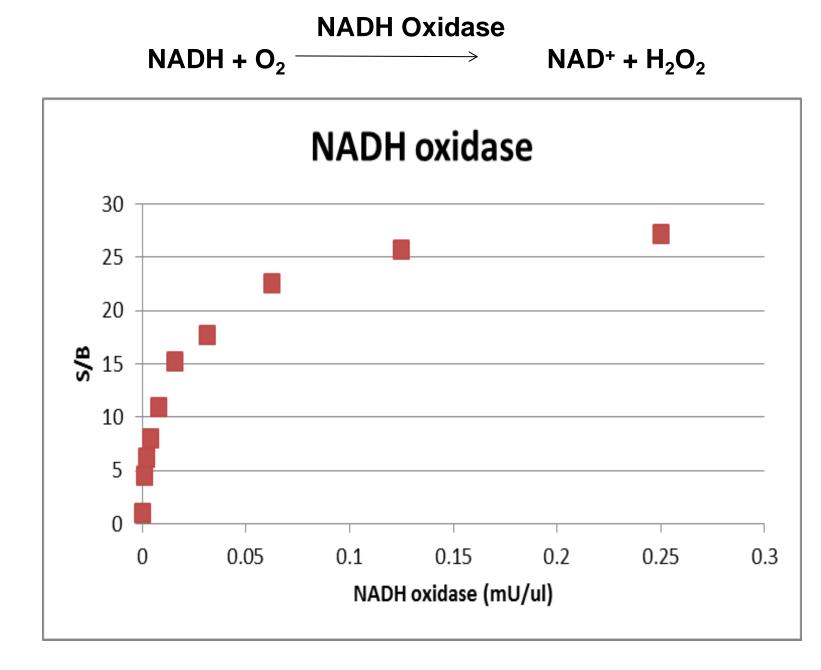


The ROS-Glo<sup>TM</sup> H<sub>2</sub>O<sub>2</sub> Substrate is added to the wells of cultured cells with test compounds.

The cells are then incubated under normal mammalian cell culture conditions.

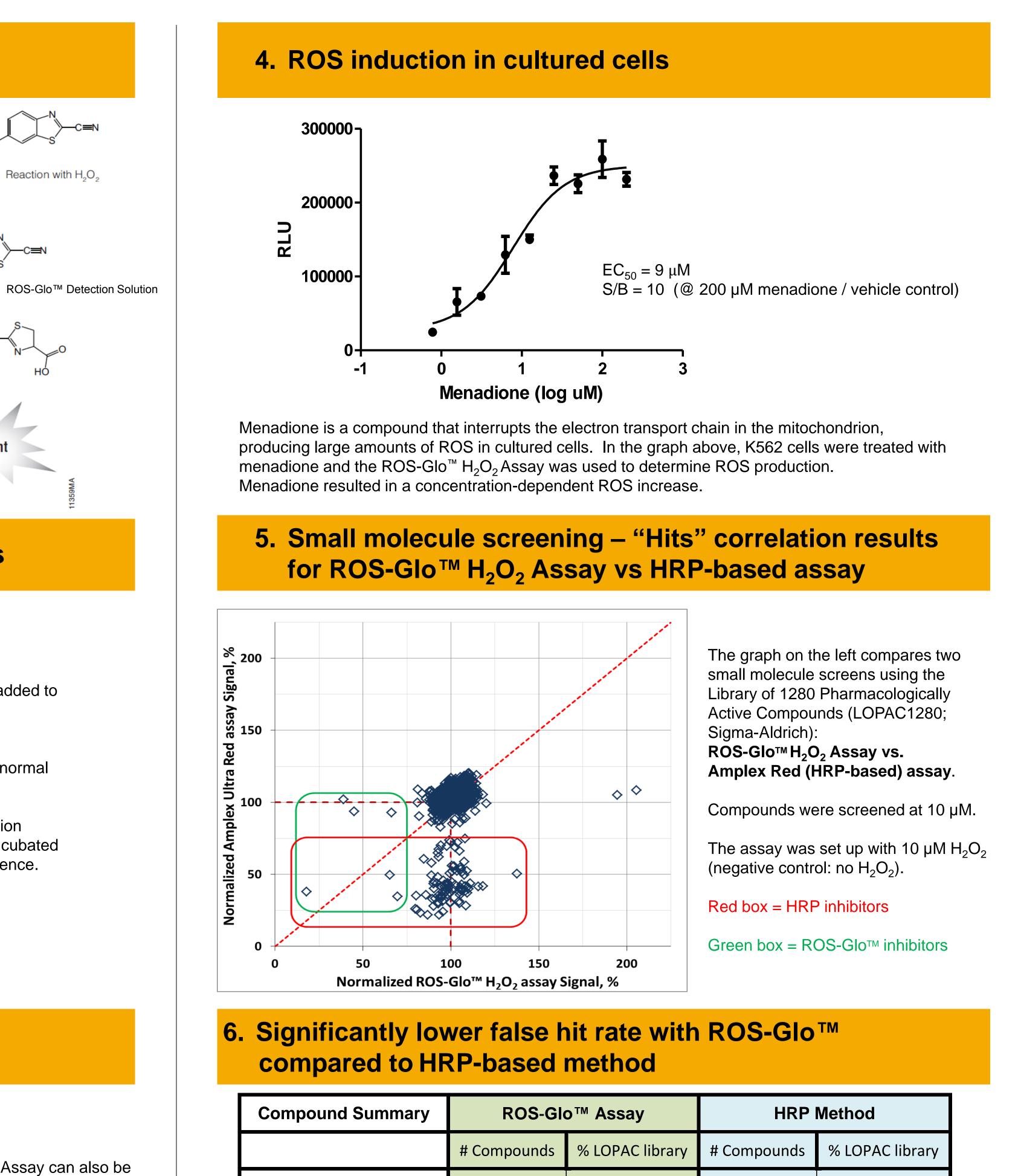
After incubation, ROS-Glo<sup>™</sup> Detection Solution is added and the plate is incubated for 20 min prior to reading luminescence.

# 3. Measuring activity of H<sub>2</sub>O<sub>2</sub>-generating enzymes



The ROS-Glo<sup>TM</sup>  $H_2O_2$  Assay can also be used to measure the activity of enzymes that generate or eliminate  $H_2O_2$ . The signal-to-background ratios from various concentrations of NADH Oxidase is shown in the graph. Once the appropriate level of NADH Oxidase is determined, the assay can then be used to identify inhibitors of the enzyme in a chemical library. The ROS-Glo<sup>™</sup> H<sub>2</sub>O<sub>2</sub> Assay limit of detection for  $H_2O_2$  is about 0.1  $\mu$ M.

#### gediminas.vidugiris@promega.com



1280

3

2

Total compounds screened

Inhibitors ≤75% activity

Inhibitors ≤50% activity

Activators ≥150% activity

reduction in  $H_2O_2$  but instead these are false hits.

generators that are missed due to HRP inhibition with Amplex Red.

100

0.5

0.2

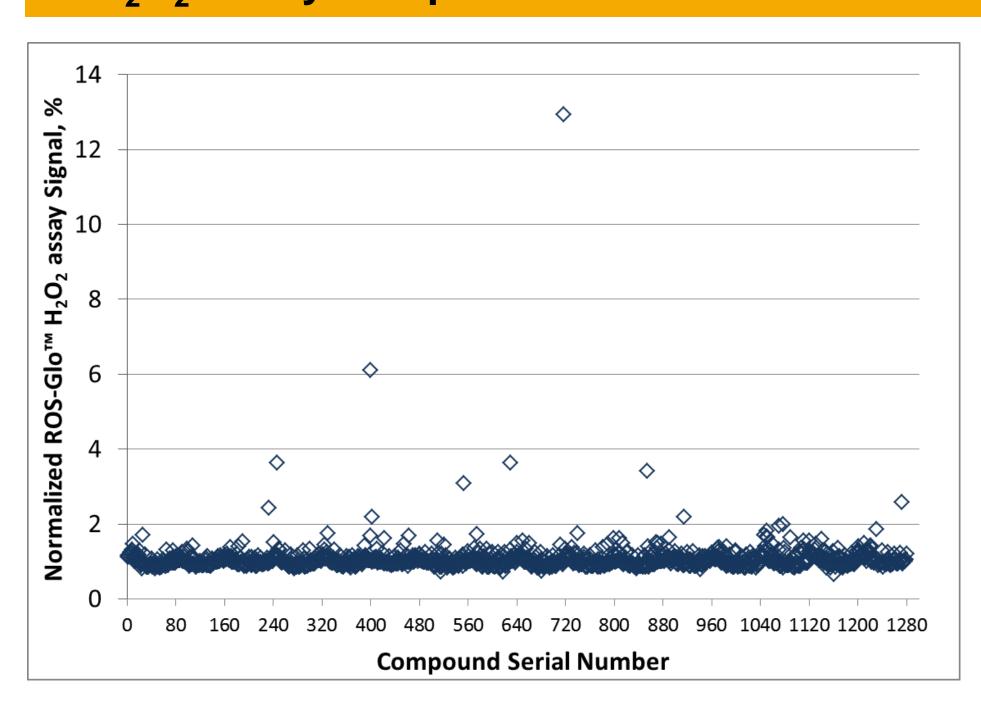
0.2

pounds	% LOPAC library
280	100
91	7.1
67	5.2
0	0

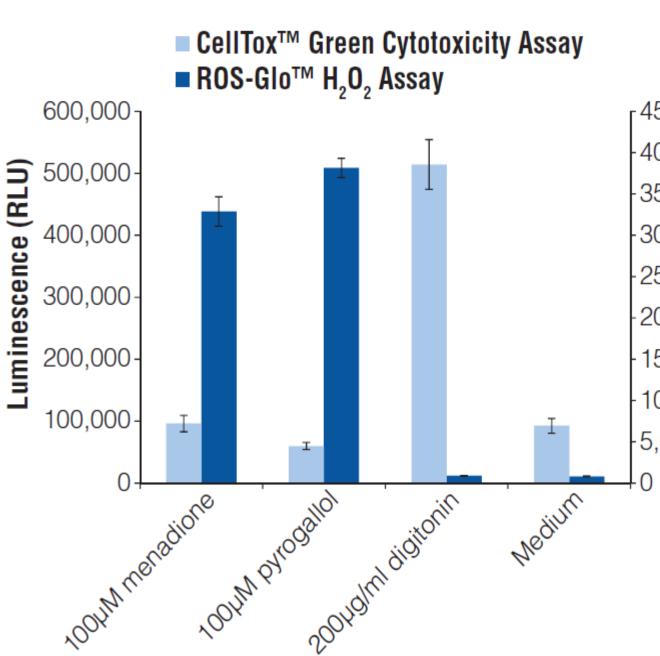
The Amplex Red assay (HRP method) uses a fluorogenic substrate to detect  $H_2O_2$  in a reaction with horseradish peroxidase (HRP). HRP reacts with numerous compounds, which appears to result in a

The ROS-Glo<sup>TM</sup> H<sub>2</sub>O<sub>2</sub> Assay uses a luminogenic probe that reacts directly with H<sub>2</sub>O<sub>2</sub> and thereby obviates the interferences associated with HRP. The signal inducers found in the ROS-Glo screen are natural ROS

#### 7. Screening of LOPAC library using the ROS-Glo<sup>™</sup> H<sub>2</sub>O<sub>2</sub> Assay – Hep G2 cells



# **8.** Multiplex ROS-Glo<sup>TM</sup> $H_2O_2$ Assay with CellTox<sup>TM</sup> Green



### 9. Summary

- > The ROS-Glo<sup>™</sup>  $H_2O_2$  Assay measures changes in the level of ROS in cultured low false hit rate and can easily be performed in multi-well plates.
- ROS-Glo can be multiplexed with a variety of assays, allowing more data to be obtained per well.
- The assay can also be used to measure the activity of enzymes that generate or eliminate  $H_2O_2$ . This allows chemical libraries to be screened for compounds that affect the activity of such enzymes as NADH Oxidase.





The ROS-Glo<sup>TM</sup> H<sub>2</sub>O<sub>2</sub> Assay was used to screen the LOPAC library using Hep G2 cells in complete media.

The compounds that increase the ROS-Glo™ signal are compounds in the LOPAC library that generate ROS in cultured mammalian cells (true nits).

r 45,000 40,000 35,000 🕤 -30,000 😇 25,000 20,000 15,000 10,000 -5,000

This multiplex allows determination of ROS production and cytotoxicity. It also allows total cell number analysis for normalization.

ROS-Glo<sup>™</sup> can be multiplexed with CellTox Green by co-applying the  $H_2O_2$ Substrate and CellTox Green dye to the cells during treatment. The fluorescent CellTox Green signal is read first, then addition of the ROS-Glo<sup>™</sup> Detection Solution allows detection of the luminescence signal which correlates with ROS levels. The ROS-Glo™ Detection Solution also lyses the cells so another fluorescent read of the CellTox Green signal will determine the total cell number and allow normalization.

mammalian cells. The method does not rely on a reaction catalyzed by HRP, has a

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