# ROS-Glo™ $H_2O_2$ Assay

INSTRUCTIONS FOR USE OF PRODUCTS G8820 AND G8821.

Quick PROTOCOL

For more information, see the *ROS-Glo™ $H_2O_2$ Assay Technical Manual* #TM391, available at: **www.promega.com/protocols**

## Cell-Based Assay Protocol

### Homogeneous Assay (Lytic Assay)

The following reagent preparation and volumes are recommended for a cell-based ROS-Glo™ $H_2O_2$ Assay in a 96-well plate. Volumes can be scaled proportionally for other plate formats. Include controls as described in Technical Manual #TM391, Section 3.C.

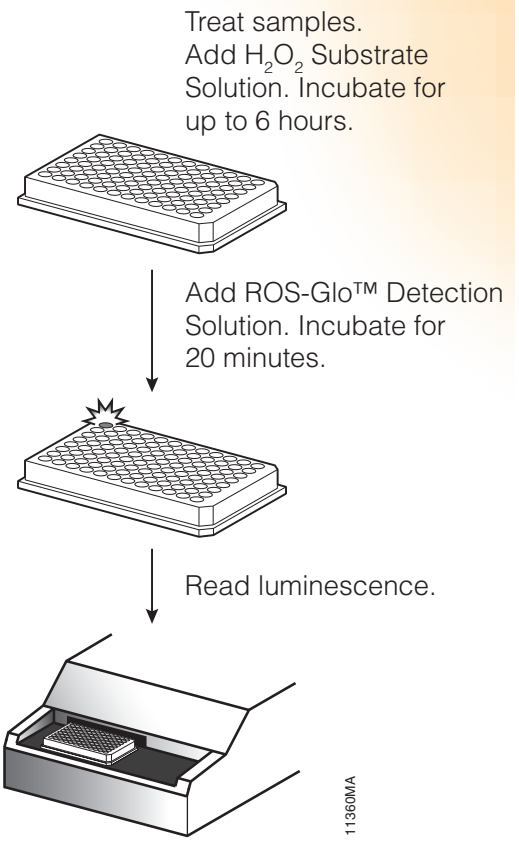
1. Plate cells at desired density in ≤80µl of medium in 96-well opaque-walled assay plates (white plates are recommended). For adherent cells allow sufficient time for attachment to plate.
2. Test compounds such as drugs or other small molecules may be added with the $H_2O_2$ Substrate Solution.

   **Note:** It is recommended to keep the final concentration of solvents such as DMSO to ≤1%.

   Add the test compound vehicle to minus-test-compound control samples (e.g., DMSO at same concentration as the test compound).
3. Thaw the $H_2O_2$ Substrate Dilution Buffer, and place it on ice. Prepare the $H_2O_2$ Substrate and test compound solution using the chilled $H_2O_2$ Substrate Dilution Buffer: Dilute the 10mM $H_2O_2$ Substrate provided in the kit to 125µM in $H_2O_2$ Substrate Dilution Buffer. Just before use, prepare an amount of $H_2O_2$ Substrate Solution sufficient for all samples including controls. For a 96-well plate, prepare the following:

| Number of Wells | $H_2O_2$ Substrate Dilution Buffer | $H_2O_2$ Substrate |
|---|---|---|
| 10 | 200µl | 2.5µl |
| 50 | 1.0ml | 12.5µl |
| 100 | 2.0ml | 25µl |

4. Add 20µl of $H_2O_2$ Substrate solution (or 20µl of combined $H_2O_2$ Substrate and test compound) to cells and mix. The final well volume will be 100µl, and the final $H_2O_2$ Substrate concentration will be 25µM.
5. Place cells in an incubator (e.g., 37°C, 5% $CO_2$ incubator) for the desired treatment time.

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## Homogeneous Assay (Lytic; continued)

**Note:** If experimental treatment time is longer than 6 hours, it is recommended to add the $H_2O_2$ Substrate for the final 6 hours of treatment. For example, if the cells are to be treated with test compound for 24 hours, add the test compound first, incubate the cells for 18 hours, then add the $H_2O_2$ Substrate Solution and return the plate to the incubator for the final 6 hours of treatment.

6. Add 100µl of ROS-Glo™ Detection Solution (see TM391, Section 3.B) to each well.
7. Incubate for 20 minutes at room temperature (22°–25°C).
8. Record relative luminescence units using a plate-reading luminometer.

## Non-Lytic Assay

The non-lytic assay preserves cells for downstream applications. Media samples are transferred to a separate plate after exposure to the $H_2O_2$ Substrate (Step 4 of Homogeneous Assay) and combined with an equal volume of ROS-Glo™ Detection Solution.

1. After Step 5 of the Homogeneous Assay Protocol, combine 50µl of media from each sample well with 50µl of ROS-Glo™ Detection Solution in a separate opaque white plate.
2. Incubate for 20 minutes at room temperature.
3. Record relative luminescence units (RLU) using a plate-reading luminometer.
4. Cells in the original sample plate can be assayed separately for other parameters, such as cell viability (see TM391, Section 5.A, Multiplexing Protocols).

## Data Analysis for Cell-Based ROS-Glo™ $H_2O_2$ Assays

Before examining various potential experimental outcomes, note this list of essential aspects of $H_2O_2$ dynamics in cell culture systems:

- $H_2O_2$ is cell membrane-permeable. When produced inside cells it diffuses into the medium, and when produced in the medium it diffuses into cells. ROS-Glo™ $H_2O_2$ Assay detects $H_2O_2$ in the plate well without regard to its source.
- Cultured cells have a strong capacity to eliminate $H_2O_2$.
- Certain compounds cause cells to produce $H_2O_2$.
- Certain compounds undergo reactions in cell culture medium that produce $H_2O_2$ independent of cells (abiotic ROS production).
- Certain cell culture media contain significant amounts of $H_2O_2$ (likely due to oxidation of medium components), and certain media contain components that react with and eliminate $H_2O_2$.

Detailed protocols and instructions can be found in the *ROS-Glo™ $H_2O_2$ Assay Technical Manual* #TM391, available online at: **www.promega.com/protocols**


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