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## Biochemical Measures of Neuropathy - Hydrogen Peroxide Assay V.2 [↗](#)

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Works for me

[dx.doi.org/10.17504/protocols.io.7rghm3w](https://doi.org/10.17504/protocols.io.7rghm3w)

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### ABSTRACT

#### Summary:

Oxidative stress is highly correlated with the metabolic changes caused by hyperglycemia. Increased levels of glucose overload mitochondria and result in the production of reactive oxygen species (ROS). In addition, the flow of excess glucose through cellular pathways decreases the cell's normal ability to detoxify ROS. As a result, the neurons and axons of the peripheral nervous system contain increased levels of ROS and decreased antioxidant capacity. The following assays are used to measure these changes in rodent models of diabetic neuropathy.

#### Diabetic Complication:



Neuropathy

### EXTERNAL LINK

<https://www.diacomp.org/shared/document.aspx?id=54&docType=Protocol>

### MATERIALS

NAME ▾

CATALOG # ▾

VENDOR ▾

[Amplex Red Hydrogen Peroxide Assay Kit](#)

A-12212

[HPLC-grade water](#)

### MATERIALS TEXT

#### Reagent Preparation:

**Amplex Red reagent:** Prepare a ~20 mM stock solution. Bring DMSO and 1 vial of Amplex Red reagent to room temp. Just prior to use dissolve the Amplex Red reagent in 200 µL DMSO. Store stock solution at -20°C, protected from light.

**Reaction Buffer (5X) (0.25M sodium phosphate, pH 7.4):** Dilute 5 mL of Reaction buffer in 20 mL of deionized water.

**HRP (Horseradish peroxidase):** Dissolve 1 vial of HRP in 1 mL of 1X Reaction Buffer (200 U/mL). After use divide remaining stock into small aliquots and store frozen at -20°C.

**20mM H<sub>2</sub>O<sub>2</sub>:** Dilute (check bottle for %) 17.9 µL H<sub>2</sub>O<sub>2</sub> (3.8%) in 982.1 µL dH<sub>2</sub>O. (Check label for exact concentration) (23 µL 3% H<sub>2</sub>O<sub>2</sub> into 977 µL dH<sub>2</sub>O) Use promptly.

**Resorufin, sodium salt:** add 1mL dH<sub>2</sub>O to a vial of resorufin solid. (2 mM stock). Store at -20°C, protected from light.

### Sample Preparation:

- 1 Prepare stock solutions above for Amplex Red Reagent, Reaction Buffer, HRP, 20 mM H<sub>2</sub>O<sub>2</sub> and Resorufin, sodium salt.
- 2 Prepare H<sub>2</sub>O<sub>2</sub> standard curve. Label tubes 1-5.  
Add 999 µL 1X Reaction Buffer to tube 2.  
Add 100 µL 1X Reaction Buffer to tubes 2 – 5.  
Add 1 µL of 20 mM H<sub>2</sub>O<sub>2</sub> working dilution prepared above to tube # 1 to produce concentrations of 20 µM.  
Take 100 µL from tube 1 and add to tube 2.  
Take 100 µL from tube 2 and add to tube 3.  
Take 100 µL from tube 3 and add to tube 4.  
Tube 5 is dH<sub>2</sub>O only.  
*(Final concentration will be twofold lower, 0 to 10 µM.)*
- 3 Dilute samples in 1X Reaction Buffer.
- 4 Pipette 100 µL of diluted standards, controls (if any) and samples into wells. (For DRG we used 25 µL)
- 5 From 20 µM stock solution of Amplex Red reagent - Prepare 400 µM dilution containing 2 U/mL HRP by adding 20 µL of Amplex Red stock solution and 100 µL of 200 U/mL HRP stock solution to 9.7 mL of 1X Reaction Buffer.
- 6 Initiate reaction by adding 100 µL from above to each well.
- 7 Place plate into Fluroskan holder and click **START**.
- 8 Take 4 readings at 15 minute intervals using 544/590 filter pairs.
- 9 Save raw data as an Excel file into the RHPx data folder. Use the naming convention RHXXXX.xls, where XXXX is the date in mmdd format.
- 10 Select Process>Organize. Choose the appropriate data to organize (usually Measure1), then click **OK**. This rearranges the data into columns.
- 11 Save organized data as an Excel file into the RHP data folder. Use the naming convention rhXXXX.xls, where XXXX is the date in mmdd format.



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