



Aug 14,
2019

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

Eva Feldman¹

¹University of Michigan - Ann Arbor

1

Works for me

[dx.doi.org/10.17504/protocols.io.3scgnaw](https://doi.org/10.17504/protocols.io.3scgnaw)

Diabetic Complications Consortium

Tech. support email: rmcindoe@augusta.edu



Lili Liang

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

[Amplex Red Hydrogen Peroxide Assay Kit](#)

CATALOG #

A-12212

VENDOR

[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 μ L 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 H₂O₂: Dilute (check bottle for %) 17.9 µL H₂O₂ (3.8%) in 982.1 µL dH₂O. (Check label for exact concentration) (23 µL 3% H₂O₂ into 977 µL dH₂O) Use promptly.

Resorufin, sodium salt: add 1mL dH₂O 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 H₂O₂ and Resorufin, sodium salt.
- 2 Prepare H₂O₂ 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 H₂O₂ 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 dH₂O 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.



This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

