Oct 10, 2025

Nitrite assay

DOI
https://dx.doi.org/10.17504/protocols.io.n2bvjejd5gk5/v1

Alfonso Diaz[1]

[1]Benemérita Universidad Autónoma de Puebla

Daniel Juarez Serrano
Benemerita Universidad Autónoma de Puebla

DOI: https://dx.doi.org/10.17504/protocols.io.n2bvjejd5gk5/v1

Protocol Citation: Alfonso Diaz 2025. Nitrite assay. **protocols.io** https://dx.doi.org/10.17504/protocols.io.n2bvjejd5gk5/v1

Protocol status: Working
We use this protocol and it's working

Created: October 09, 2025

Last Modified: October 10, 2025

Protocol Integer ID: 229487

Keywords: evaluating nitrite level, nitrite level, nitrite, griess reagent, griess method, napthylethylenediamine dihydrochloride, assay

Abstract

This protocol describes the procedures for evaluating nitrite levels using the Griess method. The Griess reagent was composed of sulfanilamide, N-1-napthylethylenediamine dihydrochloride (NED) under acidic (phosphoric acid) conditions.

Materials

Reagents

- Griess reagent (sulfanilamide, NED, phosphoric acid)
- 0.1 M nitrite standard solution
- 0.1 M phosphate-buffered saline (PBS, pH 7.4)
- Coomassie reagent (for protein assay)

Samples

- Rat prefrontal cortex tissue homogenates

Equipment

- Refrigerated centrifuge (12,500 rpm)
- Homogenizer
- 96-well microplate
- Micropipettes and sterile tips
- Spectrophotometer or plate reader (540 nm)
- Microcentrifuge tubes
- −70 °C freezer
- Ice bath

Tissue preparation

1 Rats were decapitated and the brains were immediately removed.

2 Prefrontal cortex was dissected and homogenized in 3 ml of ice-cold 0.1 M PBS, pH 7.4.

3 The homogenate was centrifuged at 12,500 rpm (4 °C).

4 The supernatant was obtained and stored at -70 °C.

Prepare reference curve in the same matrix or buffer used for experimental samples.

5 Prepare 1ml of a 100µM nitrite working solution by diluting the supplied 0.1M nitrite standard 1:1,000 in the same buffer used for the test samples.

6 Assign 3 adjacent columns (24 wells) of a 96-well plate to generate the nitrite standard reference curve. Add 50µl of the buffer into the wells in rows B–H.

7 Dispense 100µl of the 100µM nitrite solution into the 3 wells in row A.

8 Carry out 6 serial twofold dilutions (50µl per well) in triplicate down the plate to produce the nitrite standard reference curve (100, 50, 25, 12.5, 6.25, 3.13 and 1.56µM). Discard 50µl from the final dilution (1.56µM). Leave the last set of wells without nitrite (0µM).

9 Note: Each well should contain a final volume of 50µl, covering a concentration range of 0–100µM.

Nitrite Measurement (Griess Reaction)

10 Add 50 µl of each experimental sample into wells, in triplicate.

11 Add 50 µl of Sulfanilamide Solution to all wells (samples and Nitrite Standard curve).

12 Incubate for 10 min at room temperature under diminished light.

13 Add 50 µl of NED Solution to each well.

14 Incubate for 10 min at room temperature under diminished light. A purple to magenta coloration will appear rapidly.

15 Samples were examined in a spectrophotometer at 540 nm.

16 The protein content of each sample was determined by the Coomassie method. To calculate the data, µM of NO_2^- per milligram of protein was used.

Protocol references

Díaz, A., Treviño, S., Guevara, J., Muñoz-Arenas, G., Brambila, E., Espinosa, B., Moreno-Rodríguez, A., Lopez-Lopez, G., Peña-Rosas, U., Venegas, B., Handal-Silva, A., Morán-Perales, J. L., Flores, G., & Aguilar-Alonso, P. (2016). Energy Drink Administration in Combination with Alcohol Causes an Inflammatory Response and Oxidative Stress in the Hippocampus and Temporal Cortex of Rats. *Oxidative medicine and cellular longevity*, *2016*, 8725354. https://doi.org/10.1155/2016/8725354

Diaz, A., Limon, D., Chávez, R., Zenteno, E., & Guevara, J. (2012). Aβ25-35 injection into the temporal cortex induces chronic inflammation that contributes to neurodegeneration and spatial memory impairment in rats. *Journal of Alzheimer's disease : JAD*, *30*(3), 505–522. https://doi.org/10.3233/JAD-2012-111979