



Jan 15, 2026

Nitrite test with the Griess reagent system

 [PLOS One](#)

DOI

<https://dx.doi.org/10.17504/protocols.io.kqdg31ypel25/v1>

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DOI: <https://dx.doi.org/10.17504/protocols.io.kqdg31ypel25/v1>

External link: <https://doi.org/10.1371/journal.pone.0339028>

Protocol Citation: Mario Alberto Bautista-Carro 2026. Nitrite test with the Griess reagent system. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.kqdg31ypel25/v1>

Manuscript citation:

Bautista-Carro MA, Sánchez-Teoyotl P, Juárez-Serrano D, Iannitti T, Díaz A, Flores G, Morales-Medina JC (2026) Olfactory bulbectomy induces neurobiological alterations in the prefrontal cortex and hyperlocomotion in male rats. PLOS One 21(1). doi: [10.1371/journal.pone.0339028](https://doi.org/10.1371/journal.pone.0339028)



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Protocol status: Working

We use this protocol and it's working

Created: October 08, 2025

Last Modified: January 15, 2026

Protocol Integer ID: 229344

Keywords: Determination of nitrites, Spectrophotometry, Griess reaction, Nitric oxide, nitrite test with the griess reagent system, nitrite test, evaluating nitrite level, nitrite level, griess reagent, griess reagent system, nitrite, griess method, naphthylethylenediamine dihydrochloride, test

Abstract

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

Guidelines

We use a Griess reagent system.

Materials

Rodent Guillotine

Phosphate buffer solution

Laboratory centrifuge

Micropipette

96-well plate

Spectrophotometer

Griess Reagent System from Promega

Before start

Verify that the required materials and equipment are available.

Tissue preparation

- 1 Decapitate rats and immediately remove the brains.
- 2 Dissect the brain region of interest and homogenize in 3 ml of ice-cold 0.1 M phosphate buffer solution (PBS), pH 7.4.
- 3 Centrifuge the homogenate at 12,500 rpm (4 °C).
- 4 Collect the supernatant and store it at -70 °C.

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

- 5 Prepare 1ml of a 100 μ M 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 50 μ l of the buffer into the wells in rows B–H.
- 7 Dispense 100 μ l of the 100 μ M nitrite solution into the 3 wells in row A.
- 8 Carry out 6 serial twofold dilutions (50 μ l per well) in triplicate down the plate to produce the nitrite standard reference curve (100, 50, 25, 12.5, 6.25, 3.13 and 1.56 μ M). Discard 50 μ l from the final dilution (1.56 μ M). Leave the last set of wells without nitrite (0 μ M).
Note: Each well should contain a final volume of 50 μ l, covering a concentration range of 0–100 μ M.

Nitrite Measurement (Griess Reaction)

- 9 Add 50 μ l of each experimental sample into wells, in triplicate.
- 10 Add 50 μ l of Sulfanilamide Solution to all wells (samples and nitrite standard curve).

- 11 Incubate for 10 min at room temperature under diminished light.
- 12 Add 50 µl of NED Solution to each well.
- 13 Incubate for 10 min at room temperature under diminished light. A purple to magenta coloration will appear rapidly.
- 14 Measure absorbance at 540 nm using a spectrophotometer.

Nitrite Concentration Determination in Experimental Samples

- 15 Plot the average absorbance value of each Nitrite Standard concentration as a function of "Y" with nitrite concentration as a function of "X" to create a Nitrite Standard reference curve.
- 16 Calculate each experimental sample's average absorbance value. By comparing it to the Nitrite Standard reference curve, ascertain its concentration. The results are expressed in µM of NO₂ per mg of protein.

Protocol references

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