Technical Bulletin

Nitrite Assay Kit (Griess Reagent)

Catalog Number MAK367

Product Description

Nitrogen-based ions, nitrite and nitrate, are found in almost every living organism. Furthermore, endogenous nitrite levels are found in mammals and can also be obtained from dietary sources. In humans, nitrite is further metabolized to nitric oxide and other reactive nitrogen species (nitrogen oxides). The Nitrate-Nitrite- NO biochemical pathway is well known for its participation in cell signaling, hypoxia-dependent response, and regulation of blood flow. Studies suggest that nitrite is reduced to nitric oxide in the mitochondria. Specifically, myoglobin and xanthine oxidoreductase could generate NO under hypoxic conditions leading to mitochondrial respiration.

The Nitrite Assay Kit utilizes the Griess Reagent, a classic protocol for the estimation of nitrite. In the assay, nitrite is reduced to Nitrogen Oxide using Griess Reagent I. Then, Nitrogen Oxide reacts with Griess Reagent II, forming a stable product that can be detected by its absorbance at 540 nm (A_{540}). The one- step assay is simple, fast, and can detect nitrite levels as low as 1 nmol/well.

The kit is suitable for the detection of Nitric Oxide in animal tissues (liver, kidney, etc.), cell culture (adherent or suspension cells), serum, plasma, and urine.

Components

The kit is sufficient for 200 colorimetric assays in 96-well plates.

Nitrite Assay Buffer Catalog Number MAK367A	30 mL
Griess Reagent I Catalog Number MAK367B	10 mL
Griess Reagent II Catalog Number MAK367C	10 mL
Nitrite Standard Catalog Number MAK367D	1 vial

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- Corning® Spin-X® UF concentrators (Catalog Number CLS431478)
- Refrigerated microcentrifuge capable of RCF ≥10,000 × *g*

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at 2–8 °C, protected from light. Briefly centrifuge small vials prior to opening.

Preparation Instructions

Reagent Preparation

Nitrite Assay Buffer, Griess Reagent I, and Griess Reagent II: Warm to room temperature prior to use.

Nitrite Standard: Reconstitute with 100 µL of Nitrite Assay Buffer to generate a 100 mM Nitrite Standard solution. Keep on ice while in use. Reconstituted standard is stable for 4 months when stored at 2–8 °C.

Procedure

Sample Preparation

Urine: Dilute samples 10-fold using Nitrite Assay Buffer. The normal concentration of nitrite in urine is 1–20 µM.

Serum or Plasma: Deproteinize sample(s) using a Corning Spin-X UF concentrator. Centrifuge samples at 10,000 × g at 4 °C for 10 minutes. Collect filtrate and discard retentate. The normal concentration of nitrite in serum is ~2 µM.

Cell Lysate: Rapidly homogenize tissue (10 mg) or cells (1×10^6) with 100 µL of ice-cold Nitrite Assay Buffer. Chill on ice for 10 minutes. Centrifuge at 10,000 × g at 4 °C for 5 minutes and transfer the supernatant to a fresh tube. Add 10-100 µL of sample per well and adjust the volume to 100 µL with Nitrite Assay Buffer.

Notes:

- For unknown samples, test several doses to ensure the readings are within the Standard Curve range.
- For samples exhibiting significant background, prepare parallel sample well(s) as background controls.
- To remove proteins from samples, collect ultrafiltrate using a Corning Spin-X UF concentrator.

Standard Curve Preparation

Prepare a 1 mM Nitrite Standard Solution by adding 5 µL of 100 mM Nitrite Standard to 495 µL of Nitrite Assay Buffer. Prepare Nitrite Standards in desired wells of a clear flat-bottom 96-well plate according to Table 1.

Table 1.

Preparation of Nitrite Standards

Well	1 mM Nitrite Standard Solution	Nitrite Assay Buffer	Nitrite (nmol/ well)
1	0 µL	100 µL	0
2	2 µL	98 µL	2
3	4 µL	96 µL	4
4	6 µL	94 µL	6
5	8 µL	92 µL	8
6	10 µL	90 µL	10

Reaction Mix

Add both Griess Reagents and Nitrite Assay Buffer separately to each well containing Standard and test samples in order as indicated in Table 2. **Do not premix Griess Reagents prior to the experiment.**

Table 2.

Order of Reagents added to Wells

Reagent	Volume
Griess Reagent I	10 µL
Griess Reagent II	10 µL
Nitrite Assay Buffer	80 µL

Mix well and incubate at room temperature for 10 minutes. For each background correction well, add 10 µL of Griess Reagent I and 90 µL of Nitrite Assay Buffer and mix well.

Measurement

Measure absorbance at 540 nm (A_{540}) in end-point mode at room temperature. The signal is stable for one hour after Reaction Mix was added.

Results

1. Subtract the 0 Standard reading from all other readings.
2. Plot the Nitrite Standard Curve.
3. If the sample background control reading is significant, subtract the background control reading from its paired sample reading.
4. Compare the corrected ΔA540 to the Nitrite Standard Curve to get nmol of nitrite (B) in the sample well.

Nitrite Concentration (nmol/µL or mM) =

$$(B/V) \times D$$

where:
B = the Nitrite amount in the sample from Standard Curve (nmol)
V = the volume of sample added to the reaction well (µL)
D= Sample dilution factor

Figure 1.

Typical Nitrite Standard Curve

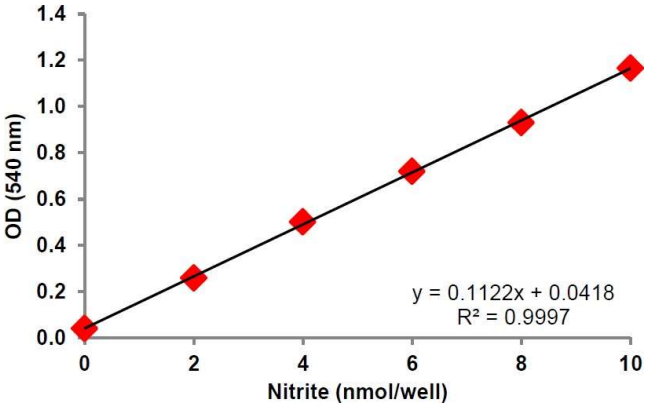
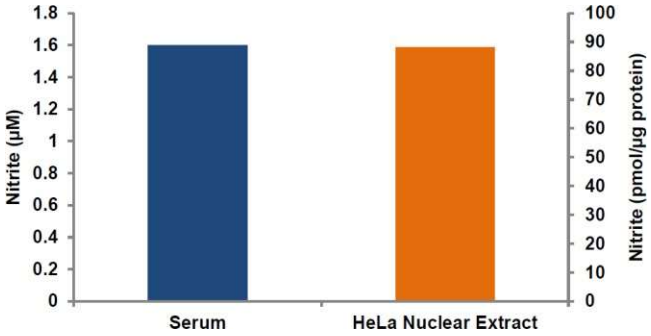


Figure 2.

Nitrite Concentration in Pooled Human Serum and HeLa Nuclear Extract



Human serum was deproteinized using a Corning Spin-X UF concentrator. Filtrate (20 µL; undiluted) was collected and assayed according to the kit procedure. HeLa nuclear cells were homogenized using Nitrite Assay Buffer (100 µg protein) and assayed as indicated.

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

Technical Service

Visit the tech service page on our web site at SigmaAldrich.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.