# Amplite® Colorimetric Nitrite Quantification Assay Kit

Catalog number: 21666
Unit size: 100 Tests

| Component | Storage | Amount (Cat No. 21666) |
|---|---|---|
| Component A: Griess Reagent | Refrigerated (2-8 °C), Minimize light exposure | 50 mL |
| Component B: Nitrite standard-50 mM | Refrigerated (2-8 °C) | 1 vial (25 µL) |

## OVERVIEW

The Amplite® Colorimetric Nitrite Quantification Assay Kit offers a sensitive colorimetric method for quantifying nitrite levels in various biological samples. Nitrite serves as a key indicator of nitric oxide (NO) production through the nitrate-nitrite-NO pathway, which plays a pivotal role in vasodilation, immune function, and cellular signaling. Precise nitrite quantification is crucial for studies investigating nitric oxide biology and its role in both physiological and pathological processes.

This kit employs a colorimetric detection strategy where nitrite reacts with a developer to produce a visible color change. The resulting color intensity, measurable using a standard absorbance microplate reader, is directly proportional to the nitrite concentration in the sample. The assay's reagents are optimized for straightforward preparation, enabling rapid, reliable results with minimal sample processing. The kit is compatible with various sample types, such as cell culture media and serum, making it an essential tool for researchers examining nitric oxide metabolism and related biological mechanisms.

## AT A GLANCE

**For 96 well plates:**

1. Prepare test samples along with serially diluted nitrite standards (75 µL).
2. Add Griess Reagent working solution (75 µL).
3. Incubate at RT for 10 minutes.
4. Measure the absorbance at 548 nm.

**For spectrophotometry cuvettes:**

1. In a 1 cm path length cuvette, mix test samples or serially diluted nitrite standards with Griess Reagents:
   - 2.6 mL of deionized water
   - 100 µL of Griess Reagent
   - 300 µL of the nitrite-containing sample
2. Incubate at RT for 10 minutes.
3. Measure the absorbance at 548 nm.

**Important note:** Thaw all the kit components at room temperature before starting the experiment.

## KEY PARAMETERS

**Absorbance microplate reader**

| | |
|---|---|
| Absorbance | 548nm |
| Recommended plate | Clear bottom white 96 well plate |

## PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/21666

**Nitrite Standard Dilution (For absorbance plate reader)**

Add 2 µL of 50 mM nitrite standard solution (component B) into 1 mL of distilled water to get 100 µM nitrite standard solution (STD7). Take 500 µL (STD7) and perform 2X serial dilutions in distilled water to get serially diluted nitrite from 50 µM, 25 µM,12.5 µM, 6.25 µM, 3.13 µM and 1.56 µM (STD6 to STD1).

## PREPARATION OF WORKING SOLUTION

**Griess Reagent Working Solution**

Add 1 mL Griess Reagents (Component A) to 6.5 mL of distilled water. Mix well, protected from light.

**Note:** Working solution should be prepared fresh for each experiment.

## SAMPLE EXPERIMENTAL PROTOCOL

**For Absorbance Microplate Reader**

1. Prepare Nitrite Standards (STD1-7), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 37.5 µL of reagent per well instead of 75 µL.
2. Add 75 µL of Griess Reagent working solution to each well of blank control, nitrite positive control and test samples. For a 384-well plate, add 37.5 µL of nitrite working solution into each well instead.
3. Incubate at RT for 10 minutes, protected from light.
4. Measure absorbance at 548 nm.

**Table 1.** Layout of Nitrite standards and test samples in a clear bottom 96-well microplate. STD= Nitrite Standards (STD1-STD7, 1.55 to 100 µM), BL=Blank Control, TS=Test Samples.

| Cell content | BL | Positive Control | TS |
|---|---|---|---|
| STD 1 | STD 1 | ... | ... |
| STD 2 | STD 2 | ... | ... |
| STD 3 | STD 3 | | |
| STD 4 | STD 4 | | |
| STD 5 | STD 5 | | |
| STD 6 | STD 6 | | |
| STD 7 | STD 7 | | |

**Note:** Nitrite concentrations in the test samples (TS) should be within the linear range of the assay (~1-100 µM).

**Table 2.** Reagent composition for each well.

| Well | Volume | Reagent |
|---|---|---|
| STD1-STD7 | 75 µL | Serial Dilutions (1.55 to 100 µM) |
| BL | 75 µL | Distilled water |
| TS | 75 µL | Test Sample |

**For Spectrophotometry Assay:**

**Nitrite Standard Dilution:**

Add 4 µL of 50 mM nitrite standard solution (component B) into 2mL of distilled water to get 100 µM nitrite standard solution (STD7). Take 1 ml (STD7) and perform 2X serial dilutions in distilled water to get serially diluted Nitrite from 50 µM, 25 µM, 12.5 µM, 6.25 µM, 3.13 µM and 1.56 µM (STD6 to STD1).

**Assay protocol:**

1. Add the following in a cuvette with 1 cm path length:
   - 2.6 mL of deionized water
   - 100 µL of Griess Reagent (Component A)
   - 300 µL of the nitrite-containing sample

   **Note:** Nitrite concentrations in the test samples (TS) should be within the linear range of the assay (1-100 µM).
2. Incubate RT for 10 min.
3. Measure absorbance at 548 nm.

## EXAMPLE DATA ANALYSIS AND FIGURES

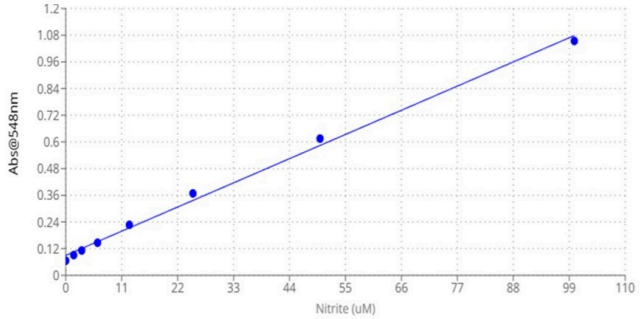


**Figure 1.** Nitrite dose response was measured with the Nitrite Quantification Assay Kit on a 96-well white microplate using a Clariostar microplate reader (BGM) at Absorbance 548nm.

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