# Amplite™ Luminometric Nitroreductase Assay Kit

Catalog number: 12470
Unit size: 100 Tests

| Component | Storage | Amount |
|---|---|---|
| Component A: NTR Substrate | Desiccated, Freeze (< -15 °C), Minimize light exposure | 1 vial |
| Component B: NTR Reaction Buffer | Freeze (< -15 °C) | 25 mL |
| Component C: NTR Reaction Enzyme | Freeze (< -15 °C) | 1 vial |
| Component D: NTR Detection Buffer | Freeze (< -15 °C) | 5 mL |
| Component E: NTR Detection Mix 1 | Freeze (< -15 °C) | 1 vial |
| Component F: NTR Detection Mix 2 | Freeze (< -15 °C) | 1 vial |
| Component G: Nitroreductase Standard | Freeze (< -15 °C) | 1 vial |
| Component H: DMSO | Freeze (< -15 °C) | 100 uL |

## OVERVIEW

Nitroreductases (NTR) are a family of evolutionarily related proteins involved in the reduction of nitrogen-containing compounds. They are absent in mammalian cells but widespread in bacteria. Nitroreductases play a crucial role in the reduction of nitroaromatic compounds via NAD(P)H-dependent reactions. NTR are also targets for developing novel antibiotics, degradation of pollutants, and cancer therapy. Although the quantification of NTR becomes extremely important for a number of biological applications, most of the current NTR assays are based on ELISA format with low sensitivity and low throughput. Amplite™ Luminometric Nitroreductase Assay Kit provides a highly sensitive and convenient method to quantify NTR activity. The kit uses a luciferin derivative that can be selectively reduced by NTR luciferin. The amount of generated luciferin is conveniently detected with the well-known luciferase detection system. The luminescent intensity generated is proportional to the NTR activity. This luminometric nitroreductase assay kit has been formulated to have minimal hands-on time and stable luminescence signal. It can detect as low as 20 ng/mL of NTR.

## AT A GLANCE

**Protocol summary**

1. Prepare NTR Substrate stock solution
2. Add NTR standards or NTR test samples (50 µL/well)
3. Add NTR Reaction working solution (50 µL/well)
4. Incubate for 60 minutes at 37 °C
5. Prepare NTR Detection working solution
6. Add NTR Detection working solution (50 µL/well)
7. Monitor luminescence intensity increase immediately

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

## KEY PARAMETERS

**Luminescence microplate reader**

Recommended plate Solid white

## PREPARATION OF STOCK SOLUTIONS

*Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.*

**1. NTR substrate stock solution (100X)**
Add 50 µL of DMSO (Component H) into the vial of NTR Substrate (Component A) to make 100X NTR substrate stock solution.

**Note** Keep away from light.

**2. NTR Reaction Enzyme stock solution (100X)**
Add 50 µL of NTR Reaction Buffer (Component B) into the vial of NTR Reaction Enzyme (Component C) to make 100X NTR Reaction Enzyme stock solution.

**Note** Keep away from light.

**3. NTR Detection Mix 1 stock solution (100X)**
Add 50 µL of NTR Detection Buffer (Component D) into the vial of NTR Detection Mix 1 (Component E) to make 100X NTR Detection Mix 1 stock solution.

**Note** Keep away from light.

**4. Nitroreductase Standard solution (250 µg/mL)**
Add 20 µL of $ddH_2O$ into the vial of Nitroreductase Standard (Component G) to make 250 µg/mL Nitroreductase Standard solution.

## PREPARATION OF STANDARD SOLUTION

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/12470

**Nitroreductase Standard solution**
Add 10 µL of 250 µg/mL Nitroreductase Standard solution to 240 µL of NTR Reaction Buffer (Component B) to generate 10 µg/mL nitroreductase standard solution (NS7). Then take 10 µg/mL nitroreductase standard solution (NS7) and perform 1:3 serial dilutions in NTR Reaction Buffer (Component B) to get serially diluted Nitroreductase standards (NS2 - NS7). Note: Diluted NTR standard solution is unstable and should be used within 4 hours.

## PREPARATION OF WORKING SOLUTION

**1. NTR Reaction working solution**
Add 50 µL of 100X NTR substrate stock and 50 µL of 100X NTR reaction enzyme stock solutions into 5 mL of NTR Reaction Buffer (Component B) to make a total volume of 5.1 mL NTR Reaction working solution.

**Note** Keep away from light.

**2. NTR Detection working solution**
Add 50 µL of 100X Detection Mix 1 and 10 µL of Detection Mix 2 (Component F) into 5 mL of NTR Detection Buffer (Component D) to make a total volume of 5.05 mL NTR Detection working solution.

**Note** Keep away from light.

## SAMPLE EXPERIMENTAL PROTOCOL

**Table 1.** Layout of NTR standards and test samples in a solid white 96-well microplate. NS=NTR standards (NS7-NS1, 0.001 to 10 µg/mL); BL=Blank Control; TS=Test Samples

| BL | BL | TS | TS |
|---|---|---|---|
| NS1 | NS1 | ... | ... |
| NS2 | NS2 | ... | ... |
| NS3 | NS3 | | |
| NS4 | NS4 | | |

| | | | |
|---|---|---|---|
| NS5 | NS5 | | |
| NS6 | NS6 | | |
| NS7 | NS7 | | |

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

| Well | Volume | Reagent |
|---|---|---|
| NS1-NS7 | 50 µL | Serial Dilutions (10 to 0.001 µg/mL) |
| BL | 50 µL | NTR Reaction Buffer (Component B) |
| TS | 50 µL | Test Sample |

1. Prepare NTR standards (NS), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 12.5 µL of reagent per well instead of 50 µL.

   **Note** Treat cells or tissue samples as desired.

2. Add 50 µL of NTR Reaction working solution to each well of NTR standard, blank control, and test samples. For a 384-well plate, add 12.5 µL of NTR Reaction working solution into each well instead.

3. Incubate the reaction for 60 minutes at 37 °C, protected from light.

4. Add 50 µL of NTR Detection working solution to each well to make the total assay volume 150 µL/well. For a 384-well plate, add 12.5 µL of Detection working solution into each well instead, for a total volume of 37.5 µL/well.

5. Monitor the luminescence increase immediately with a luminescence microplate reader.

## EXAMPLE DATA ANALYSIS AND FIGURES

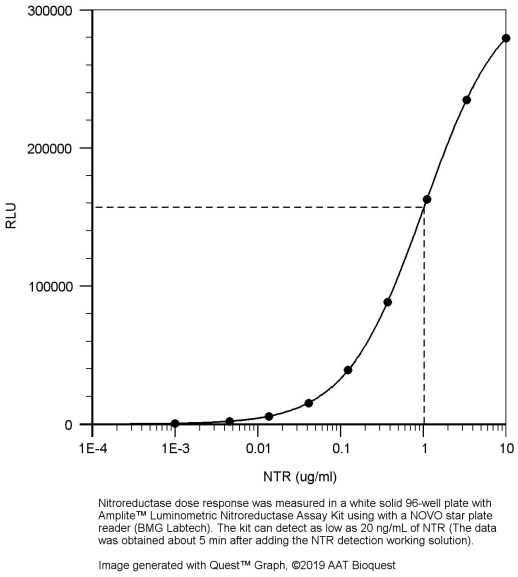


**Figure 1.** Nitroreductase dose response was measured in a white solid 96-well plate with Amplite™ Luminometric Nitroreductase Assay Kit using with a NOVO star plate reader (BMG Labtech). The kit can detect as low as 20 ng/mL of NTR (The data was obtained about 5 min after adding the NTR detection working solution).

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