ab324120 – Nitroreductase Assay Kit (Luminometric)

A highly sensitive and convenient method to quantify Nitroreductase (NTR) activity.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab324120

Storage and Stability: Store kit at -20°C in the dark immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components.

Materials Supplied

Item	Quantity	Storage Condition
NTR Substrate	1 vial	-20°C (Store in the dark)
NTR Reaction Buffer	25 mL	-20°C
NTR Reaction Enzyme	1 vial	-20°C
NTR Detection Buffer	5 mL	-20°C
NTR Detection Mix 1	1 vial	-20°C
NTR Detection Mix 2	1 vial	-20°C
Nitroreductase Standard	1 vial	-20°C
DMSO	100 µL	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Luminescence microplate reader
- Solid white plates
- ddH_2O

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 kit components at room temperature before starting the experiment.

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.

NTR substrate stock solution (100X)

1. Add 50 µL of DMSO into the vial of NTR Substrate to make 100X NTR substrate stock solution.

***Note**: Keep away from light.*

NTR Reaction Enzyme stock solution (100X)

1. Add 50 µL of NTR Reaction Buffer into the vial of NTR Reaction Enzyme to make 100X NTR Reaction Enzyme stock solution.

***Note**: Keep away from light.*

NTR Detection Mix 1 stock solution (100X)

1. Add 50 µL of NTR Detection Buffer into the vial of NTR Detection Mix 1 to make 100X NTR Detection Mix 1 stock solution.

***Note**: Keep away from light.*

Nitroreductase Standard solution (250 µg/mL)

1. Add 20 µL of ddH_2O into the vial of Nitroreductase Standard to make 250 µg/mL Nitroreductase Standard solution.

Preparation of Standard Solution

Nitroreductase Standard solution

1. Add 10 µL of 250 µg/mL Nitroreductase Standard solution to 240 µL of NTR Reaction Buffer 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 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 Solutions

NTR Reaction working solution

1. 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 to make a total volume of 5.1 mL NTR Reaction working solution.

***Note**: Keep away from light.*

NTR Detection working solution

1. Add 50 µL of 100X Detection Mix 1 and 10 µL of Detection Mix 2 into 5 mL of NTR Detection Buffer to make a total volume of 5.06 mL NTR Detection working solution.

***Note**: Keep away from light.*

Experimental Protocol

BL	BL	TS	TS
NS1	NS1	…	…
NS2	NS2	…	…
NS3	NS3		
NS4	NS4		
NS5	NS5		
NS6	NS6		
NS7	NS7		

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.

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

Table 2. Reagent composition for each well of a 96-well microplate.

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.

For technical support contact information, visit: www.abcam.com/contactus

Version 1 | 2025-05-06