ab284567 – Nitric Oxide Cell-Based HTS Assay Kit (Fluorometric)

For the quantitative measurement of Nitric Oxide Mammalian cell culture samples.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
www.abcam.com/ab284567

Storage and Stability: Store kit at -20°C immediately upon receipt.

Materials Supplied

Item	Quantity	Storage Condition
NO Assay Buffer	50 mL	-20°C
NO Inhibitor (DPI, 1mM)	20 µL	-20°C
NO Staining Dye	20 µL	-20°C
NO Standard (100 µM)	25 µL	-20°C

Materials Required, Not Supplied

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

- 96-well plates (sterile, white, or black plate with clear flat bottom)
- Multi-well spectrophotometer (Ex/Em = 488/532 nm)
- NO-producing primary cells or cell lines
- Cell-Culture Media
- 37°C Incubator with 5% CO_2

Reagents Preparation

NO Assay Buffer: Warm to 37°C before use. Use within 6 months.

NO Staining Dye and NO Standard: Light sensitive. Do not expose to direct-intense light. Aliquot and store at -20°C. Avoid multiple freeze/thaws. Use within 6 months.

NO Inhibitor (DPI): Avoid multiple freeze/thaw. Use within 6 months.

Sample Preparation

Cell Culture:

1. Grow cells in appropriate media and culture conditions. Adherent cells should be cultured to ~80% confluency. For both adherent and suspension cells, harvest cells and centrifuge at 1,000 x g, for 5 min. Resuspend the cell pellet in NO Assay Buffer and count the number of cells using a hemocytometer or an automated cell counter. Re-suspend cells in 1 ml of media at the concentration of 5 x 106 cells/ml. For 96 well-plate, add 1 ml of re-suspended cells to 9 ml of media. Mix gently by pipetting and add 100 µl of cell mixture to each well to get 50,000 cells per well.

Δ Notes:

a) For cells in suspension, after initial seeding, it is important to centrifuge the entire plate at 1,000 x g for 5 minutes, every time, before removing, replacing with any media or solutions.
b) Prepare at least 1 blank well without cells or media for background control (Dye-only) assessment.

Assay Procedure

NO Inhibitor Control (IC) and Test Compounds (TC):

1. For Inhibitor Control (IC), add 4 µl of DPI Inhibitor stock (1mM) to 500 µl of pre-warmed media concentration (1:125 dilution). Add 100 µl of diluted DPI mixture to 100 µl of pre-existing media in each well to make 200 µl total volume (4 µM DPI final concentration).
2. For Test Compounds (TC) and Vehicle Solvent Control (SC), dissolve test compounds in proper solvent(s) to produce 100X stock solutions. To make a master mix (for every 5 wells), add 12 µl of each test compound stock (100X) or 12 µl of Vehicle Solvent to 588 µl of media. Add 100 µl of media mixture with Test Compounds or Solvent to well to make to make 200 µl total volume (1x TC final concentration).
3. Pre-incubate the compounds with cells for 1 h at 37°C

Δ Notes:

a) Pre-incubation time with inhibitors or activators may vary, depending on the test compounds.
b) Final solvent concentration should be minimized to avoid solvent toxicity to cells. DMSO has been shown to have negligible effects on cell viability at a concentration of ≤ 2%.

Background Controls (BC) Preparation:

Prepare the following BC conditions (for calculations, choose the higher RFU measurement between them as your background):

- Cell-only control (CC) well: Add 100 µl of fresh media to 100 µl pre-existing media in each well to make 200 µl total volume.
- Dye-only control (DC) well: make sure the well is empty and without cells or media. Proceed to next step.

Standard curve:

1. Add 5 µl of the NO Standard stock solution (100 µM) to 95 µl NO assay buffer to generate 5 µM working solution.
2. Add 0, 2, 4, 6, 8, 10 µl of the diluted Standard to 6 consecutive blank wells (without cells) to generate 0, 10, 20, 30, 40, 50 pmol/well respectively. Bring the volume of each well to 50 µl with Assay Buffer.

Staining dye:

1. Pre-warm NO Assay buffer to 37°C. To make a master mix, for every 25 wells, add 3 µl of Staining Dye Stock to 1.5 ml of Assay Buffer (1:500 dilution).
2. Remove the media from each well by vacuum aspiration, and add 50 µl of Staining Dye diluted solution to wells containing IC, TC, SC and DC. For CC: Add 50 µl of NO Assay Buffer without staining dye.
3. Incubate the entire plate for 1 h at 37°C in the dark. No additional wash is needed after this step. The plate is ready for measurement after incubation.

Measurement

Measure fluorescence (RFU) by plate reader (Ex/Em = 488/532 nm). Top-read setting is recommended.

Calculation

1. For Standard Curve, subtract 0 Standard reading from all readings and plot the Standard Curve.
2. For samples, subtract the background control reading (from step VII-3) from all sample readings.
3. Apply ΔRFU to the Standard curve to get the amount of NO (pmoles) generated during the reaction.

Δ Note: 1 pmol of Fluorescent Triazole= 1 pmol of NO.

$$B = Nitric\ Oxide\ (pmol) = \frac{[\Delta\ RFU(TC - BC)]}{\text{Slope of standard curve}}$$

Where: **TC** = test compound
BC = Background Control

Technical Support

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