AB308317– Nitric Oxide Synthase Activity Assay Kit II (Colorimetric)

For the detection of NOS activity in various sample types.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: abcam website

Storage and Stability

Store the kit at -80 °C, protected from light. Once opened, store the kit components as recommended. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

Materials Supplied

Item	Quantity	Storage Condition
NOS Assay Buffer	25 ml	4°C or -20°C
NOS Lysis Buffer	25 ml	-20°C
NOS Substrate	0.5 ml	-20°C
NOS Cofactor 1	1 vial	-20°C
NOS Cofactor 2 (Avoid light)	0.1 ml	-20°C
Nitrate Reductase	1 vial	-20°C
NOS (Positive Control)	50 µl	-80°C
Enhancer (Avoid light)	1 vial	-20°C
Nitrite Standard	1 vial	4°C
Griess Reagent 1	10 ml	4°C
Griess Reagent 2 (Avoid light)	10 ml	4°C

Materials Required, Not Supplied

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

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer
- Protease Inhibitor Cocktail

Reagent Preparation

NOS Assay Buffer: Bring to room temperature (RT) before use. Store at 4 °C or -20 °C.

NOS Lysis Buffer: Ready to use. Store at 4 °C or -20 °C.

NOS Substrate: Ready to use. Divide into aliquots and store at -20 °C. Avoid repeated freeze/thaw. Keep on ice while in use.

NOS Cofactor 1: Reconstitute the vial in 300 µl of dH_2O to prepare 10 mM NOS Cofactor 1 working solution. Divide into aliquots and store at -20 °C. Avoid repeated freeze-thaw cycles. Keep on ice while in use.

NOS Cofactor 2 (25X): Divide into aliquots and store at -20 °C. Avoid repeated freeze/thaw cycles. Prepare 1X NOS Cofactor 2 working solution with dH_2O just before use. Keep on ice while in use.

Nitrate Reductase: Reconstitute the vial in 1.1 ml NOS Assay Buffer. Divide into aliquots and store at -20 °C. Avoid repeated freeze-thaw cycles. Keep on ice while in use.

NOS (Positive Control): Divide into aliquots and store at -80 °C. Avoid repeated freeze-thaw cycles. During use, keep the solution on ice at all times since the enzyme loses activity at higher temperatures.

Enhancer: Reconstitute the vial in 1.2 ml NOS Assay buffer. Keep on ice during use. Store at -20°C.

Nitrite Standard: Reconstitute the vial in 100 µl NOS Assay Buffer. Vortex & mix well to generate a 100 mM Nitrite Standard solution. Store at 4 °C when not in use. Do not freeze. The reconstituted Nitrite Standard solution is stable for 4 months when stored at 4 °C.

Griess Reagents 1 and Griess Reagent 2: Ready to use. Store at 4 °C.

Nitric Oxide Synthase Activity Assay Protocol:

Sample Preparation:

Rinse the tissue and transfer ~100 mg of fresh or frozen tissue (stored at -80 °C) to a pre-chilled tube. Add 200-300 µl cold NOS Lysis Buffer containing protease inhibitor cocktail and thoroughly homogenize the tissue on ice. To prepare the cell extract, add 200-300 µl cold NOS Lysis Buffer containing protease inhibitor cocktail (not provided) to fresh or frozen cells (2-5 x 10^6 cells) and homogenize to disrupt the cells. Centrifuge the tissue or the cell homogenate at 10,000 x *g*, 4 °C for 10 min. Transfer the clear supernatant to a fresh pre-chilled tube and keep on ice. Measure the protein concentration using BCA or any preferred protein assay. Use the lysates immediately for the NOS activity assay. Add 30-60 µl (200-400 µg protein) of cell or tissue homogenate or purified protein into the desired wells of a 96-well plate. For Positive Control, Add 5 µl of NOS (Positive Control) into the desired well(s). Adjust the volume of samples and Positive Control wells to 57.5 µl/well with NOS Assay Buffer.

Δ Note:

a. *We recommend using the tissue or cell homogenate immediately to measure the NOS activity. If desired, snap freeze the lysate and store at -80 °C.*

b. *For Unknown Samples, we suggest doing a pilot experiment and testing several amounts to ensure that the readings are within the Nitrite Standard Curve range.*

c. *Optional: For samples with high background, prepare a parallel sample well(s) containing same amount of sample labelled as Sample Background Control(s).*

Nitrite Standard Preparation:

Add 5 µl of reconstituted 100 mM Nitrite Standard to 495 µl NOS Assay Buffer to generate 1 mM Nitrite Standard solution. Add 0, 2, 4, 6, 8, and 10 µl of 1 mM Nitrite Standard solution into a series of wells in a 96-well plate to generate 0, 2, 4, 6, 8 and 10 nmol/well Nitrite Standard. Adjust the volume to 57.5 µl/well with NOS Assay Buffer.

Reaction Mix Preparation:

Prepare enough Reaction Mix for the number of wells (Standards, Positive Control and sample) to be analyzed. For each well, prepare 40 µl Mix:

	Reaction Mix	Background Mix
NOS Assay Buffer	10 µl	15 µl
NOS Cofactor 2 (1x)	20 µl	20 µl
NOS Substrate	5 µl	-

Nitrate Reductase	5 µl	5 µl

Mix and add 40 µl of the Reaction Mix to Standard, Positive Control, and Sample wells. Add 40 µl of Background Mix to the Sample Background Control wells. Add 2.5 µl of 10 mM NOS Cofactor 1 working solution to all the wells including Sample, Sample Background Control, Standards and Positive Control and mix well. Incubate the plate at 37 °C for 20- 30 min. After incubation, add 95 µl of NOS Assay Buffer to all wells followed by the addition of 5 µl of Enhancer into each well. Mix and incubate the plate at RT for 10 min.

Measurement:

Add 50 µl of Griess Reagent 1 and 50 µl of Griess Reagent 2 to all the wells including Sample, Sample Background Control, Standard, and Positive Control. Mix and incubate for 10 min. Measure the absorbance at 540 nm in a microplate reader in endpoint mode.

Calculation:

Subtract 0 Standard reading from all readings. Plot the Nitrite Standard Curve. If the Sample Background Control reading is significant, subtract the Sample Background Control reading from the Sample readings to get the corrected Sample reading. Apply the corrected Sample reading to the Standard Curve to get B nmoles of Nitrite generated during the reaction.

Sample Nitric Oxide Synthase Specific Activity $= \frac{B}{T \; x \; C}$ **= nmol/min/mg = mU/mg**

Where, **B** is the Nitrite amount in the sample well from the Standard Curve (nmol).
T is the reaction time (min.)
C is the amount of protein (mg)

Unit Definition:

One unit of NOS activity is the amount of enzyme required to yield 1.0 µmol of nitric oxide/minute at 37 °C.

Technical Support

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