CheKine™ Nitric Oxide (NO) Colorimetric Assay Kit

Cat #: KTB1400 Size: 48 T/96 T/192 T

	Nitric Oxide (NO) Colorimetric Assay Kit (Colorimetric)		
REF	**Cat #:** KTB1400	LOT	**Lot #:** Refer to product label
	Applicable samples: Tissues/Cells, Plasma, Serum, Urine (and other Biological Fluids)		
	Storage: Storage at -20℃ for 12 months, protected from light		

Assay Principle

Nitric oxide (NO) is a reactive radical that plays an important role in many key physiological functions such as neurotransmission, immune response and apoptosis. NO is synthesized from L-arginine by NO synthase (NOS). It has been identified as an endothelial derived relaxation factor (EDRF) and antiplatelet substance. It serves as a neurotransmitter derived from a neutrophil and a cytotoxic substance from an activated macrophage. Although NO's molecular action in the biological system is very versatile, the most important role of NO is the activation of guanylate cyclase. CheKine™ Nitric Oxide (NO) Colorimetric Assay Kit provides a convenient tool for sensitive detection of NO level. The principle is that Since NO is oxidized to Nitrite and Nitrate, it is common to quantitate total NO^{2-}/NO^{3-} as a measure of NO level. The Nitric Oxide Assay Kit is designed to accurately measure NO production following reduction of Nitrate to Nitrite by using improved Griess method. The Griess assay's mechanism is summarized as the azo coupling between diazonium species, which are produced from sulfanilamide with NO^{2-} and N-(1-naphthyl) ethylenediamine dihydrochloride, resulting in a colorimetric (540 nm) product proportional to the NO metabolite present.

Materials Supplied and Storage Conditions

Kit components	Size			Storage conditions
	48 T	96 T	192 T	
$NaNO_2$ Standard (1 M)	1 mL	1 mL	1 mL	-20℃, protected from light
Griess Reagent Ⅰ	3 mL	6 mL	12 mL	4℃, protected from light
Griess Reagent Ⅱ	3 mL	6 mL	12 mL	4℃, protected from light
VCl_3 Reagent	6 mL	12 mL	24 mL	4℃, protected from light
$ZnSO_4$	0.5 mL	1 mL	2 mL	4℃, protected from light

Materials Required but Not Supplied

• Microplate Reader capable of measuring absorbance at OD540 nm

- 96 well Plate with clear flat bottom, Precision pipettes, Disposable pipette tips
- Distilled or Deionized water
- Dounce homogenizer

Reagent Preparation

$NaNO_2$ Standard (1 M): Equilibrate to room temperature and protect from light during the assay. Aliquot so that you have enough volume to perform the desired number of assays. Store aliquots at -20℃, and protected from light.

Griess Reagent I: Ready to use as supplied. Equilibrate to room temperature and protected from light during the assay. Store at 4℃, and protected from light.

Griess Reagent II : Ready to use as supplied. Equilibrate to room temperature and protected from light during the assay. Store at 4℃, and protected from light.

VCl_3 Reagent: Ready to use as supplied. Equilibrate to room temperature and protected from light during the assay. Store at 4℃, and protected from light.

$ZnSO_4$: Ready to use as supplied. Equilibrate to room temperature and protected from light during the assay. Store at 4℃, and protected from light.

Standard preparation:

Prepare 10 mM of $NaNO_2$ Standard stock solution Ⅰ by diluting 10 µL $NaNO_2$ Standard (1 M) into 990 µL PBS (pH 7.4). Prepare 100 µM of $NaNO_2$ Standard stock solution Ⅱ by diluting 10 µL 10 mM of $NaNO_2$ Standard into 990 µL PBS (pH 7.4). Using 100 µM standard stock solution Ⅱ , prepare standard curve dilution as described in the table:

	100 µM Standard stock solution Ⅱ (µL)	pH 7.4 PBS (µL)	Concentration (µM)
Std.1	200	0	100
Std.2	100	100	50
Std.3	40	160	20
Std.4	20	180	10
Std.5	10	190	5
Std.6	4	196	2
Std.7	2	198	1
Blank	0	200	0

Note: Always prepare a fresh set of standards per use. Diluted standard solution is unstable and must be used within 4 hours.

Sample Preparation

1. Tissues or Cells sample: Weigh 0.1 g tissue or collect 5×10^6 Cells add 1 mL PBS (pH 7.4) and homogenize. Centrifuge at 14,000 rpm for 10 minutes at 4℃. Use supernatant for NO assay.

2. Plasma, Serum and Urine (and other Biological Fluids): Tested directly by adding samples to the microplate wells. Samples that need to conduct deproteinization include Serum, Plasma, whole Blood, Cell culture media containing FBS, Tissue or Cell lysates. Urine and Saliva do not need deproteinization.

Deproteination: Mix 150 µL sample with 8 µL $ZnSO_4$ in 1.5 mL tubes. Vortex and then centrifuge at 14,000 rpm for 10 min at 4℃. Transfer 100 µL of the clear supernatant to a clean tube.

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month. If samples need to be deproteinated, 150 µL of each standard should be prepared and also be treated with $ZnSO_4$ to eliminate the need for a dilution factor.

Assay Procedure

1. The Working Reagent Preparation: For each well of reaction, prepare 204 μL Working Reagent by mixing 104 μL VCl_3 Reagent, 50 μL Griess Reagent Ⅰ and 50 μL Griess Reagent Ⅱ. Fresh reconstitution is recommended.
2. Reaction: Add 100 μL of diluted Standard and Sample to separate, labeled ependorf tubes (We recommend that Samples be measured in at least duplicate). Then add 200 μL Working Reagent to each Sample and Standard tube, mix well.
3. Incubate the reaction for 30 minutes at 37℃.
4. Briefly centrifuge the reaction tubes to pellet any condensation and transfer 250 μL of each reaction to separate wells in a 96 well plate. Read OD at 540 nm.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If the OD_{Sample} is higher than 1.0, please further dilute the sample with PBS (pH 7.4). Pay attention to multiply by the dilution factor when calculating the result.

Data Analysis

Subtract blank OD from the standards and samples OD values, calculate $\Delta OD_{Standard}=OD_{Standard}-OD_{Blank}$, $\Delta OD_{Sample}=OD_{Sample}-OD_{Blank}$. With the concentration of the standard Solution as the x-axis and the $\Delta OD_{Standard}$ as the y-axis, draw the standard curve. Substitute the ΔOD_{Sample} into the equation to obtain the x value (μM). It's the NO content.

Where: $OD_{Standard}$: the OD of different concentration Standard; OD_{Sample}: the OD of Sample; OD_{Blank}: the OD of Blank.

Conversions: 1 mg/dL NO equals 333 μM, 0.001% or 10 ppm.

Note: Antioxidants and nucleophiles (e.g. β-mercaptoethanol, glutathione, dithiothreitol and cysteine) may interfere with this assay. Avoid using these compounds during sample preparation.

Typical data

Typical standard curve-data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

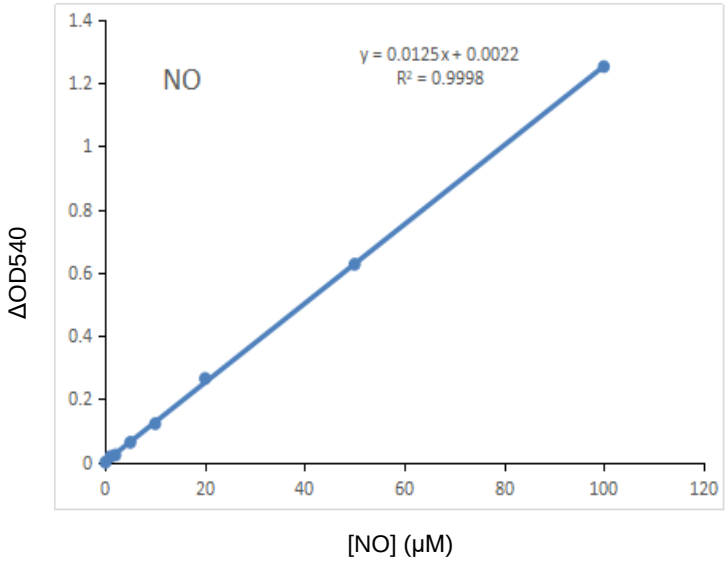

Figure 1. Standard Curve of NO in 96-well plate assay.

Recommended Products

Catalog No.	Product Name
KTB1010	CheKine™ Coenzyme II NADP(H) Assay Kit (Colorimetric)
KTB1020	CheKine™ Coenzyme I NAD(H) Colorimetric Assay Kit (Colorimetric)
KTB1030	CheKine™ Superoxide Dismutases (SOD) Assay Kit (Colorimetric)
KTB1070	CheKine™ Xanthine Oxidase Assay Kit (Colorimetric)

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.