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CheKine™ Micro Nitric Oxide (NO) Assay Kit

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

	Micro Nitric Oxide (NO) Assay Kit		
REF	**Cat #:** KTB1400	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant 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™ Micro Nitric Oxide (NO) 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	
$NaNO_2$ Standard (1 M)	1 mL	1 mL	-20℃, protected from light
Griess Reagent Ⅰ	4 mL	8 mL	4℃, protected from light
Griess Reagent Ⅱ	4 mL	8 mL	4℃, protected from light
VCl_3 Reagent	7.5 mL	15 mL	4℃, protected from light
$ZnSO_4$	0.6 mL	1.2 mL	4℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 540 nm
- Incubator
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips

• Deionized water, PBS (pH 7.4)
• Dounce homogenizer (for tissues samples)

Reagent Preparation

$NaNO_2$ Standard (1 M): Equilibrate to room temperature and protect from light during the assay. 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:

Num.	100 μM Standard stock solution Ⅱ (μL)	PBS (pH 7.4) (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 fresh standards per use. Diluted standard solution is unstable and must be used within 4 h.

Sample Preparation

1. Animal and Plant Tissues or Cell samples: 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 min 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. Antioxidants and nucleophiles (e.g. β-mercaptoethanol, glutathione, dithiothreitol and cysteine) may interfere with this assay. Avoid using these compounds during sample preparation.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 540 nm, Visible

spectrophotometer was returned to zero with deionized water.

2. 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. Working Reagent is freshly prepared.

3. Add 100 μL diluted Standards and Sample to separately labeled EP tubes (We recommend that Samples be measured in at least duplicate). Then add 200 μL Working Reagent to each Sample and Standard tube, then mix well.

4. Incubate the reaction for 30 min at 37℃.

5. Briefly centrifuge the reaction tubes to pellet any condensation and transfer 250 μL of each reaction to separate wells in a 96 -well plate or microglass cuvette. Read A at 540 nm. and recorded as A_{Blank}, $A_{Standard}$ and A_{Sample}, respectively. Subtract A_{Blank} from $A_{Standard}$ and A_{Sample}, that is, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$, $\Delta A_{Sample}=A_{Sample}-A_{Blank}$.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If the A_{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. Increase the sample size if the ΔA_{Sample} value is below 0.005.

Data Analysis

With the concentration of the Standard Solution as the x-axis and the $\Delta A_{Standard}$ as the y-axis, draw the standard curve. Substitute the ΔA_{Sample} into the equation to obtain the x value (μM). It's the NO content.

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

Typical Data

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

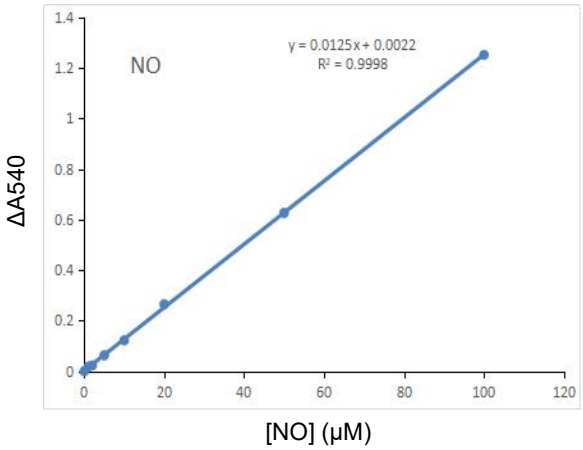

Figure 1. Standard Curve of NO in 96-well plate assay.

Recommended Products

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

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.