A013-2-1 96T

Nitric Oxide (NO) Assay Kit (microplate format)

Organism Species: *Pan-species* (General)

Instruction manual

FOR RESEARCH USE ONLY

NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

12th Edition

[INTENDED USE]

The kit is a one step method for the in vitro quantitative measurement of NO in blood serum(plasma), tissue and other biological fluids.

Nitric oxide (NO, endothelium - derived relaxing factor) , is a free radical with extremely high reactivity in vivo, and it plays a role as a second messenger and neurotransmitter. It is an effector molecule which has wide physiological actions such as relax vascular smooth muscle, restrain platelet aggregation, regulate cerebral blood flow, mediate cellular toxic effect and immunolo - regulation，participated in learning and memory, atherosclerosis, etc. Abnormal NO producing relates to development of some diseases. As the result, medical researchers pay more attention to NO researches.

[REAGENTS AND MATERIALS PROVIDED]

Reagents	Quantity(96T)	Reagents	Quantity(96T)
Reagent 1	1×20ml	Reagent 5	1×3ml
Reagent 2	1×10ml	2mmol/L Sodium nitrite Standard	1×1ml
Reagent 3	1×10ml	96-well strip plate	1
Reagent 4	1×3ml	Instruction manual	1

Note：2mmol/L standard solution should be diluted before the measurement to the concentration of 20μmol/L with double distilled water.

[STORAGE OF THE KITS]

1. Reagent 1: Solution, pretreatment agent for samples.Can be stored at 4°C for 12 months.
2. Reagent 2: Solution, pretreatment agent for samples.Can be stored at 4°C for 12 months.
3. Reagent 3: Solution, chromogenic agent. Can be stored at 4°C away from light for 12 months.
4. Reagent 4: Solution, chromogenic agent.Can be stored at 4°C away from light for 12 months.
5. Reagent 5: Solution, chromogenic agent. Can be stored at 4°C for 12 months.
6. 2mmol/L Sodium nitrite Standard: Solution. Can be stored at 4°C for 12 months.

[REAGENT PREPARATION]

1. **Developer**: R3:R4:R5=2.5:1:1, prepare when you need, and store at 4°C,you cannot use when the color deepen.
2. **Standard**: Dilute the 2 mmol/L Sodium nitrite Standard with distilled water at 1:39, 1:79, 1:99, 1:159, 1:319, 1:639 to prepare 0.05mmol/L, 0.025mmol/L, 0.02mmol/L, 0.0125mmol/L, 0.00625mmol/L, 0.003125mmol/L standard solution.

[SAMPLE PREPARATION]

1. **Blood serum(plasma) Pretreatment:**

Take blood serum(plasma) stock solution to determine.

2. **Tissue Pretreatment:**

Animal tissue: Measure the weight of tissue sample accurately, make tissue homogenate at the weight - volume ratio of 1:9 with normal saline, centrifuge at 2500~3000rpm/min for 10 minutes, take supernatant for assay.

Plant tissue: Measure the weight of tissue sample accurately, make tissue homogenate at the weight - volume ratio of 1:9 with 0.1mol/L pH7~7.4 PBS, centrifuge at 4000rpm/min for 10 minutes, take supernatant for assay.

Cultured cells: Culture the cell with six - orifice plate, if you want to determine the protein content of cell culture fluid, take it to detect direct; if you want to determine the protein content of cells, digest the cell with trypsogen (or scrape the cell directly),take out the cell and fluid and put into test tube, centrifuge at 1000～3000rpm/min for 5 minutes, abandon the supernatant and keep the precipitation cell. Add 1ml normal saline tootle and mix well(wash out the trypsogenand culture solution), centrifuge at 1000~3000rpm/min for 5 minutes, abandon the supernatant and keep the precipitation cell, add 0.2~0.3ml normal saline to make homogenate.(you can homogenize by following 4 ways:① Cell lysate;② Supersonic wave;③ Hand movement;④ Glass homogenizer, 5~10 seconds once ,and interval 20 seconds, grind 3~4 times),take homogenate to assay.

Note: Shake uniformity before take samples.

[ASSAY PROCEDURE]

1. Pre-Processing for standard

Dilute the 2 mmol/L Sodium nitrite Standard with distilled water at 1:39, 1:79, 1:99, 1:159, 1:319, 1:639 to prepare 0.05mmol/L, 0.025mmol/L, 0.02mmol/L, 0.0125mmol/L, 0.00625mmol/L, 0.003125mmol/L standard solution.

2. Pre-Processing for sample

Blood serum(plasma):

Take blood serum(plasma) stock solution 100ul, add reagent 1 200μl,mix well, add reagent 2 100μl,mix well and standing for 10 minutes, then centrifuge at 3500~4000rpm/min 15min,take supernatant 160μl for assay.

Tissue:

Take 10% homogenate supernatant 300μl,add reagent 1 200μl, mix well, add reagent 2 100μl, mix well and standing for 10 minutes, and then centrifuge at 3500~4000rpm/min 15min to take 160μl supernatant for assay.

3. Operation table

	Blank tube	Standard tube	Sample tube
Double distilled (ml)	0.16		
20 μmol/L Sodium nitrite Standard（ml）		0.16	
Supernatant（ml）			0.16
Developer（ml）	0.08	0.08	0.08
Mix well，standing 15 minutes，measure the OD value at 550nm			

[TEST PRINCIPLE]

NO has a very short half-life, and blood NO is produced by vascular endothelial cells, vascular smooth muscle cell, blood platelets, macrophages, as the forms of nitrates and nitrites, so it is able to determine NO concentration by nitrates and nitrites' concentrations determination indirectly.

[CALCULATION OF RESULTS]

Use the readings for each standard, control, and samples to subtract the zero standard optical density. Construct

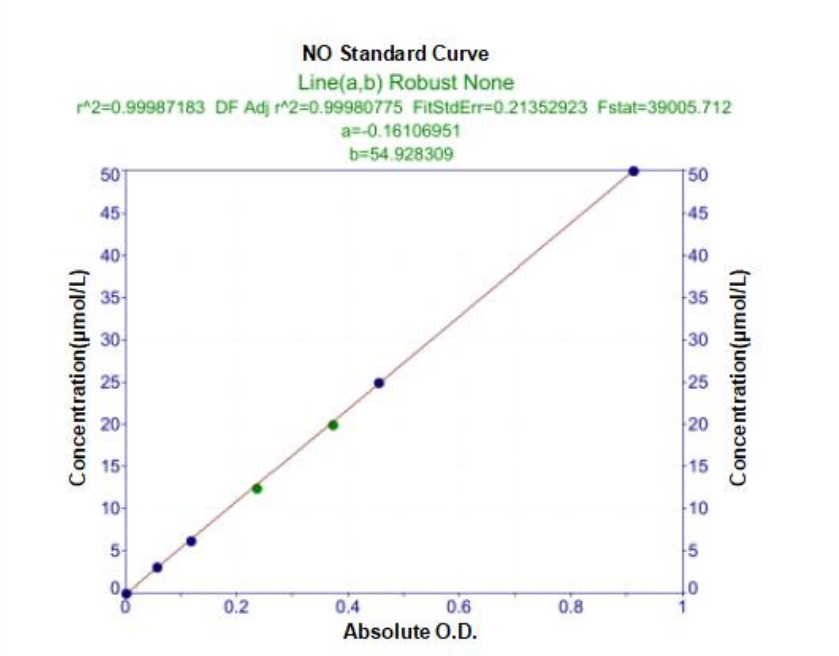
a standard curve by plotting the O.D. and concentration for each standard and draw a best fit curve through the points on the graph or create a standard curve on log-log graph paper with NO concentration on the y-axis and absorbance on the x-axis. Using some plot software, for instance, curve expert 1.30, is also recommended. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

[TYPICAL DATA]

In order to make the calculation easier, we plot the O.D. value of the standard (X-axis) against the known concentration of the standard (Y-axis), although concentration is the independent variable and O.D. value is the dependent variable. However, the O.D. values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), plotting log of the data to establish standard curve for each test is recommended. Typical standard curve below is provided for reference only.

1. Standard Curve

Standard Concentration（μmol/L）	Assay OD	Absolute OD
0	0.0406	0
3.125	0.0975	0.0569
6.25	0.1581	0.1175
12.5	0.2766	0.236
20	0.4117	0.3711
25	0.4957	0.4551
50	0.9523	0.9117



Typical Standard Curve for Nitric Oxide Assay

2. **Blood serum**

Blood serum calculation formula:

$$NO\ Content = \frac{\text{AssayOD - BlankOD}}{\text{StandardOD - BlankOD}} \times \text{standard concentration} \times \text{Dilution times}$$

$(\mu mol/L)$ $20\mu mol/L$ (4)

Note：Dilution times=Dilution before determine

Example:

Take blood serum 0.1ml and operate according the pre‐processing, OD of Blank is 0.0406, OD of Standard is 0.4117,OD of Assay is 0.0795.Calculate with the formula：

$$NO\ Content = \frac{Assary OD - Blank OD}{Standard OD - Blank OD} \times Standard\ concentration \times Dliution\ times$$

$(\mu mol/L)$ $20\mu mol/L$ (4)

$$= \frac{0.0795 - 0.0406}{0.4117 - 0.0406} \times 20 \times 4 = 8.38\mu mol/L$$

3. Tissue

Tissue calculation formula:

$$NO\ Content = \frac{Assary OD - Blank OD}{Standard OD - Blank OD} \times Standard\ concentration \times \text{Dilution times} \div \text{Samples' protein concentration}$$

$(\mu\mu mol/gprt)$ $20\mu mol/L$ (2) (gprot/L)

Note：Dilution times=Dilution before determine

Example:

Take 10% mouse liver homogenized supernatant 0.3ml and operate according the pre‐processing, OD of Blank is 0.0406,OD of Standard is 0.4117,OD of Assay is 0.0543.Atthe same time, the protein content of 10% mouse liver homogenate is 11.6574 gprot/L. Calculation shown below：

$$NO\ Content = \frac{\text{AssayOD - BlankOD}}{\text{StandardOD - BlankOD}} \times Standard\ concentration \times Dilution\ times \div \text{Samples' protein concentration}$$

$(\mu\mu mol/gprt)$ $20\mu mol/L$ (2) (gprot/L)

$$= \frac{0.0543 - 0.0406}{0.4117 - 0.0406} \times 20 \times 2 \div 11.6574 = 0.127\mu mol/gprot$$

[Technology Parameter]

Serial Number	Item	Request
1	Kit Detection Limit	0.2μmol/L
2	Kit Intra‐assay CV	2.11%
3	Kit Intra‐assay CV	5.65%
4	Kit Recovery Rate	99.5%
5	Linear range 0.2~50μmol/L	R2=0.999
6	Wavelength Choice Limit	530nm~570nm

[ASSAY PROCEDURE SUMMARY]

1. Prepare all reagents, samples and standards;
2. Add reagent to each tube according to the operation table;
3. Mix well，standing 15 minutes，measure the OD value at 550nm.

[IMPORTANT NOTE]

1. Severity following the operating instruction.
2. Supernant must be clear, were it turbid, it is advised to centrifuge again.
3. Hemolysis and turbid blood serum will affect the results.
4. Blood and tissue can keep 3 days at 4~5°C.The lower temperature, the longer expiration date. It can be kept 1~2 months at -20°C.
5. Please refer to blood serum(plasma) to detect the culture fluid and calculate.