Product Information

Total Nitric Oxide Assay Kit

Catalog Number: T6027

Product Size: 50T / 200T (96-well plate)

Contents:

Component	50T	200T
A. Sample diluent	4 mL	16 mL
B. NADPH	0.5 mg	2 mg
C. FAD	0.5 mL	2×1 mL
D. Nitrate Reductase	250 μL	1 mL
E. LDH Buffer	0.5 mL	2×1 mL
F. LDH	0.5 mL	2×1 mL
G. 1M $NaNO_2$	250 μL	1 mL
H. Griess Reagent I	2.5 mL	10 mL
I. Griess Reagent II	2.5 mL	10 mL

Storage

Stored at -20℃, The kit is stable for at least one year from the date it is received. NADPH, Nitrate Reductase, $NaNO_2$, Griess reagent I and Griess reagent II should be stored in dark. The solution of NADPH should be divided into several parts and stored at -70℃.

Description

As an important gas signal molecule, NO widely exists in organisms and various tissues, and participates in many biological effects and physiological and pathological processes. NO has free radicals, which makes its chemical properties very active, and rapidly oxidizes to form the mixture of nitrite and nitrate. Based on the principle of Griess reaction, this kit adopts the optimized reaction conditions to reduce nitrate to nitrite by nitrate reductase, so as to achieve the purpose of detecting the content of total NO.

The kit can detect the content of NO in cell lysate, tissue lysate, cell or tissue culture medium, serum, plasma or urine.

Protocol

1. Sample treatment

The sample containing high concentration protein, such as serum or cell culture medium containing high concentration serum, may produce precipitation after adding Griess reagent I. If precipitation occurs, the sample can be heated in boiling water bath for 5min to denature the protein, and then 12,000g centrifugation for 5min. The supernatant is used for subsequent determination. The cell or tissue samples

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can be lysed with corresponding lysate. For urine samples, it is usually necessary to dilute 10-50 times with water. Heparin anticoagulant plasma should not be used. Heparin anticoagulant plasma will react with Griess reagent to produce precipitation.

2. Dilute the standard: dilute 1M $NaNO_2$ into 1 μM, 10 μM, 20 μM, 40 μM, 80 μM, 100 μM with the solution used in the preparation or dilution of the sample.

2.1 Take 1 μL of 1M $NaNO_2$ standard was added to the diluent of 1 mL sample to obtain 1 mM $NaNO_2$ solution.

2.2 Take 10 μL of 1 mM $NaNO_2$ solution was added to the diluent of 90 μL sample to obtain 100 μM $NaNO_2$ solution.

2.3 Take 80 μL of 100μM $NaNO_2$ solution was added to the diluent of 20 μL sample to obtain 80 μM $NaNO_2$ solution.

2.4 Take 80 μM $NaNO_2$ solution and dilute it with the diluent used for the sample with equal volume to obtain 40 μM $NaNO_2$ solution. Dilute with the same method to obtain 10 μM and 20 μM $NaNO_2$ solution.

2.5 Take 5 μL of 20 μM $NaNO_2$ solution was added to the diluent of 95 μL sample to obtain 1 μM $NaNO_2$ solution.

If the sample is serum, it can be simply diluted with PBS, normal saline and other appropriate solutions. The diluted standard should be prepared and used now, and should not be used after frozen storage.

3. Preparation of reagents

3.1 Add about 1.2mL double distilled water or Milli-Q water into 2mg NADPH, mix and dissolve it, prepare 2 mM NADPH, put it on ice bath for use, the remaining NADPH solution must be immediately sub packed and frozen at -70℃.

3.2 FAD can be stored at -20℃ or -70℃ after proper sub packaging, and used on ice bath.

3.3 Nitrate Reductase and LDH should be placed on the ice bath for use (please keep it at -20℃ immediately after use). Griess reagent I and Griess reagent II were restored to room temperature before use.

4. Refer to the table below, add the standard, sample and detection reagent in turn and carry out the corresponding test.

5. The concentration of NO in the sample was calculated according to the standard curve.

Standards, samples and test reagents

	Control	Standard	Samples
Standard	-	60μL	
Sample	-	-	x μL
Detection reagent	60 μL	-	(60-x) μL
NADPH (2mM)	5 μL	5 μL	5 μL
FAD	10 μL	10 μL	10 μL
Nitrate Reductase	5 μL	5 μL	5 μL
After mixing, incubate at 37℃ for 30 min			
LDH Buffer	10 μL	10 μL	10 μL
LDH	10 μL	10 μL	10 μL
After mixing, incubate at 37 ℃ for 30 min			
Griess Reagent I	50 μL	50 μL	50 μL
Griess Reagent II	50 μL	50 μL	50 μL
After mixing, incubate at room temperature (20-30 ℃) for 10min, and determine the absorbance value at 540 nm			

Notes

1. If Griess reagent I contains precipitate when taken out, it can

be placed in a 37 ℃ water bath until the precipitate dissolves.

2. For the culture medium containing high concentration of nitrate, it is necessary to replace it with other appropriate medium, such as DMEM, MEM, F12, etc., or HBSS or PBS, etc. before detecting NO. Because higher concentration of nitrate will interfere with the result.

3. Due to the reduction reaction in the detection process, all oxidation or reduction reagents that affect the reduction reaction should be avoided, such as common reducing agents DTT and mercaptoethanol.

4. The reaction must be conducted away from light, and bubbles should be avoided in each detection hole when adding reagent each time, so as not to interfere with the test results.

5. The upper limit of sample dosage is 60 μL, and 40 μL is usually enough for serum, plasma or tissue homogenate. When the sample is less than 60 μL, the volume of different samples should be consistent, and the insufficient volume should be supplemented with the solution used for preparing or diluting the sample.

6. 2-3 wells with 200 μL water or PBS can be set as negative control at the same time. Only water or PBS is added in these 2-3 holes, and no other reagents are added.

7. When testing, if there is no 540 nm filter, 520-560 nm filter can also be used.

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